Comparative preliminary phytochemical investigation and antioxidant analysis of *Acacia mearnsii, Bracteantha bracteata, Grevillea robusta and Passiflora tarminiana*

BY

KP. ARTHI

Reg.No.17PBO004

A Dissertation submitted to the

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore – 641-043.

In Partial Fulfillment of the Requirements for the

DEGREE OF MASTER OF SCIENCE IN BOTANY

APRIL 2019

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25/4/19

Signature of the

Head of Department

25/04/19

Signature of supervisor

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Acknowledgement

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Introduction

INTRODUCTION

Since ancient times, long before prehistoric period, people have been using plants and its products not only as food source but also as medicine. The practice of using plants as medicine is very ancient and reliable. India, which has a wide range of tropical and agro climatic conditions, possesses rich floristic wealth and diversified resources of medicinal plants. Recently WHO (World Health Organisation) estimated that 80 percent of people worldwide rely on plants for some of their primary health care.

Huge advances is being done and carried out in the field of modern medicine, but still most people from developing world is relying on traditional medicine to treat illness and disease, due to its affordable price and less side effects. Plants usage as medicine are currently in considerable significance view due to their special attributes as a large source of therapeutic phytochemicals that may lead to the development of novel drugs. Presence of naturally occurring phytochemicals in plants is due to their defence mechanism to protect themselves from various diseases, to withstand extreme high temperature and UV light. Based on the protective function, physical characteristics and chemical characteristics more than 4,000 phytochemicals have been have been catalogued and classified. These phytochemicals from various unique sources (leaf, bark, stem, fruits, flowers etc.,) possess interesting bioactive compounds and medicinal properties.

Plants use a diverse set of secondary biochemical pathways not to fulfill their primary metabolic needs in energy and biosynthetic products, but to generate a number of secondary metabolites called phytochemicals (Harborne, 1993; Gershenzon, 1994; Reymond *et al*., 2000; Hermsmeier *et al*., 2001 and Kennedy and Wightman, 2011). They are non-nutritive, biologically active compounds naturally found in plants.

Most of the phytochemicals from plant sources such as phenolics and flavonoids have been reported to have positive impact on health and prevention of diseases (Venugopal and Liu, 2012). Modern Mediterranean and DASH (Dietary Approaches to Stop Hypertension) incorporate a phytochemicals rich diet from fruit and vegetable sources as the plant based diet has shown to extend life span in Okinawan people, that has the highest number of centenarians (Willcox *et al.,* 2007 and Willcox *et al*., 2009). Increase in research and industrial application of medicinal plants is due to the interest in utilizing natural sources

in the development and formulation of skin products, as an alternative to conventional drugs and synthetic products (Mukherjee *et al*., 2011).

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. Free radicals are highly reactive molecules containing one or more unpaired electron; they donate or take electron from other molecule in an attempt to pair their electron and generate a stable species (Aliyu *et al*., 2012). In order to maintain healthy biological system, maintenance of balance between oxidation and antioxidation is very important (Tiwari, 2001). Thus, intake of anti-oxidant rich vegetables, fruits etc., is highly recommended.

Antioxidants are both natural and synthetic compounds able to scavenge free radicals and inhibit oxidation processes (Hayat *et al*., 2010). Synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, are currently available and have been suspected to cause or prompt negative health effects. The above mentioned widely used synthetic antioxidants used in food industry may be responsible for liver damage and carcinogenesis (Grice, 1988). In order to avoid the side effects, strong restrictions have been placed on their application. At present there is an increasing trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity (Barlow, 1990). Besides well known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices, some natural antioxidant (e.g. rosemary and sage) are already exploited commercially either as antioxidant additives or a nutritional supplements (Schuler, 1990).

Many other plant species have been investigated in the search for novel antioxidants (Chu, 2000; Koleva *et al*., 2002; Mantle *et al*., 2000 and Oke and Hamburger, 2002) but generally there is still a demand to find more information concerning the antioxidant potential of plant species. It has been mentioned that the antioxidant activity of plants might be due to their phenolic compounds (Cook and Samman, 1996). Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel, 1995). Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski *et al*., 1987).

Thus the present work has been undertaken by taking four commonly available plants from Ooty, with the following objectives

- To obtain extract from leaf and stem of *Acacia mearnsii, Bracteantha bracteata, Grevillea robusta and Passiflora tarminiana* using Ethyl Acetate, Methanol and Water.

- To investigate the presence or absence of 25 phytochemicals qualitatively in *Acacia mearnsii, Bracteantha bracteata, Grevillea robusta and Passiflora tarminiana* in ethyl acetate, methanolic and aqueous extracts of leaf and stem

- To assess and compare the quantitative phytochemicals in *Acacia mearnsii, Bracteantha bracteata, Grevillea robusta and Passiflora tarminiana* in the three extracts of leaf and stem.

- To assess and compare the antioxidant activity in *Acacia mearnsii, Bracteantha bracteata, Grevillea robusta and Passiflora tarminiana* in extracts of leaf and stem

- To do correlation study between the total phenol present and DPPH activity.

Review of literature

Review of Literature

Importance of plants is well known to all. Recently the awareness that plant kingdom is a treasure house of potential drugs and their importance of being used as medicines are increasing. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. (Dewick, 1996). According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of safer drugs. About 80% of individuals from developed countries are using traditional medicines, which has compounds derived from medicinal plants. Such plants in traditional medicines and also in our daily life are to be investigated for understanding their properties, safety, and efficiency better (Phillipson and Wright, 1996).

Plants have been used for medicinal purposes throughout human history, and the first pharmaceuticals (that is quantified doses of medicinal compounds as opposed to crude extracts of plant material) were derived from medicinal plants (Black, 1997). Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines. The annual global export value of 50,000 to 70,000 types of plants with suspected medicinal properties was estimated to be US$2.2 billion in 2012 (Medicinal and aromatic plants trade programme, 2017).

India and China are two of the largest countries which have the richest flora of medicinal plants (Raven, 1998). India is considered as one of the Mega Diversity Centre in the world having wide variety of plants and rich in ethnic diversity. The climatic and ecological diversity create a foundation for very rich plant diversity Studies on wild edible plants have been carried out by various workers in India (Kaul *et al*, 1982; Nagar, 1985; Sebastian & Bhandari, 1990; Bora & Pande, 1996; Girach *et al*, 1988; ; Islami & Jha, 2001; Sharma & Singh, 2001; Lalramnghinglova, 2002; Patole & Jain, 2002; Pundir & Singh, 2002; Chakraborty, 2003; Krishna Prasad *et al*, 2003 Kumer, 2003; Kulkarni *et al*, 2003; Devi, 2003; Nandanakunjidam, 2003; Narasimhan, 2003; Prasad *et al*, 2003). Indian region has long been recognized as a centre of agro- diversity (Vavilo, 1951; Zeven and de Wet, 1982). It contains over 5% of the world's diversity though it covers only 2% of the earth's surface but it is one of the biodiversity hotspots of the richest and highly endangered eco- regions of the world (Myers *et al*.,2000).

Traditional medicinal plants use in India is about 4000 years old (Sivasankari, 2013). India has a rich flora which is also unique and widely distributed throughout the country. The

Indian subcontinent is endowed with rich and diverse local health tradition, which is equally matched with rich and diverse plant genetic source (Pushpangadan, 1995). It has 17,000 species of higher plants; among these 7500 species have medicinal properties (Shiva, 1996). Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions from ancient period through traditional methods practiced such as Ayurveda, Unani and Siddha. Ayurveda which is the oldest medical system in Indian sub-continent reported that approximately 2000 plant species are widely used as a medicine (Prajapati *et al*., 2003).

Tamilnadu is one of the most botanized areas of South India, where the use of plant as medicine is still of great importance. The medicinal properties of many medicinal plants were studied by several workers in Tamilnadu (Rajadurai *et al.,* 2009; Duraipandiyan and Ignacimuthu, 2011; Alagesaboopath, 2011 and Bhaskar and Samant, 2012). Plants have been used as folk remedies and ethnobotanical literature has described the usage of plant extracts, infusions and powders for centuries for diseases now known to be of viral origin (Berghe *et al*., 1986).

Nilgiri district popularly known as “The Blue Mountains” or “blue hills” (Neelam- blue and Giri- hill or mountain) is the smallest districts in the state of Tamilnadu which lies between 11º, 12’ and 11º 43 N and 76º14’ and 77º 1’ E in the Western Ghats. It is situated in tropics with a climate varying between sub-tropical and temperate with an annual rain fall of this district ranges from 1600-1800 mm. It is one of the most spectacular natural mountains ranges in South India; the Nilgiris are situated at the confluence of the Western and Eastern Ghats as the Sahayadri Hills, comes under Nilgiri Biosphere Reserve.

Allopathic medicine may cure many diseases but it has high prices and also causes side effects. Many people return to herbal medicines because it has fewer side effects when compared to allopathy (Kala, 2005). Newman *et al*., (2003) was stated that approximately 119 chemical substances obtained from 90 plant species were important drugs in one or more countries. Natural products serve as an important source of new pharmaceutical agents today. Novel drugs plays many important roles like anti-infectives, anti-inflammatories and drugs used in the treatment of cardiovascular and metabolic diseases are often natural products, natural product derivatives, or inspired by natural products (Butler, 2005; Newman *et al*., 2003; Newman and Cragg, 2007; Wilson and Danishefsky, 2007).

Several plant species are used by many ethnic groups for the treatment of various ailments ranging from minor infections to dysentery, skin diseases, asthma, malaria and a horde of other indications (Dhar *et al*., 1968; Perumal Samy and Ignacimuthu, 1998, 2000; Dahanukar *et al*., 2000). Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs are obtained from natural sources or semi synthetic derivatives of natural products used in the traditional systems of medicine (Dewick, 1996). Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced in the market are obtained from natural or semi synthetic resources (Phillipson and Wright 1996).

Medicinal plants are mostly used in pharmaceutical field for the wide range of substances present in plants which have been used to treat chronic as well as infectious diseases (Arunkumar and Muthuselvam, 2009). Medicinal plants are not only used in pharmaceuticals, it is also used in cosmetics, and neutraceuticals. Due to this wide range of uses medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases (Stary and Hans, 1998). The plant extracts have been developed and proposed for use as antimicrobial substances (Campo *et al*., 2000). Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases. Medicinal herbs practiced in traditional folk medicine in India were screened for the presence of antibacterial activity (Ghosh *et al*., 2007). Plants which are found in common places as weeds, hedge plants, shade trees etc., also have certain medicinal properties in them.

Acacia mearnsii

Synonyms

Acacia decurrens auct. non Willd

Acacia mollissima sensu auct.

Albizia mearnsii De Wild

Racoperma (De Wild)

Acacia mearnsii belongs to the family Fabaceae. The Fabaceae or Leguminosae commonly

known as the legume, pea, or bean family, are a large and economically important family of flowering plants. It includes trees, shrubs and herbaceous plants perennials or annuals, which are easily recognized by their fruits (legume) and their compound, stipulated leaves. The group is widely distributed and is the third-largest land plant family in terms of number of species. The largest genera are *Astragalus* (over 2,400 species), *Acacia* (over 950 species), *Indigofera* (around 700 species), *Crotalaria* (around 700 species), and *Mimosa* (around 500 species), which contain around 9.4% of all flowering plant species (Magalion, and Sanderson, 2001).

The genus *Acacia* contains over 1000 species, which is one of the largest genus in the Fabaceae family contains over 3000 species (Stevens, 2018). *Acacia* species are commonly known as 'Babool' kikar or Indian gum Arabic tree in India. Pedanius Dioscorides (a Greek physician and 'father of botany', ca. 40 to 90 A.D.) described a preparation of extracts from the leaves and fruit pods of a plant in his book on "Medicinal Material" and he called it 'akakia', from which the name of the genus Acacia originated. Acacia was formally adopted by Miller in a paper describing 24 African and American species (Miller, 1754). Prior to Miller, the name *Acacia* had been widely used in pre-Linnean literature (Ross, 1980).

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There are some 1350 species of *Acacia* found throughout the world (Stevens, 2018) and approximately 1000 species found in Australia, where it is commonly known as "wattle". Gamble, (1918) have reported more than 40 species of this genus in India in his 'Flora of Madras Presidency. *Acacia* is the largest genus of vascular plants in Australia. The genus *Acacia* can divided be into three genera: *Acacia* -161 species, *Senegalia* -231 species and *Racosperma* 960 species (Pedley, 1986).

The Belgian naturalist Émile Auguste Joseph De Wildeman first described *A. mearnsii* De Wild. in 1925 (De Wildeman). This species was first collected by E.A. Mearns from a cultivated specimen in East Africa (Tame *et al*). *A. mearnsii* is native to south-eastern

Australia and commonly found on Tasmania, Western Australia, India, Hawaiian Islands, Africa, the Caribbean, East Asia, Europe, Sri Lanka, North America, New Zealand, South America and Southeast Asia (World Wide Wattle. *Acacia mearnsii* De Wild. *A.mearnsii* is a fast growing, evergreen pioneer leguminous tree that is native to Australia which was introduced to South Africa over 150 years ago primarily for tanning industry (Sherry, 1971; Xiang *et al*., 2006).

A. mearnsii is a fast-growing evergreen leguminous shrub or small tree. At maturity the species generally attains 5-15 m in height and 10-35 cm dbh with form that varies from multi-stemmed shrubs to singlestemmed trees with straight boles 2 m long (Sherry, 1971). it is very rare to find any individuals taller than 20 m or larger than 45 cm dbh. The stems of *A. mearnsii* do not have spines or prickles, the leaves are bipinnate on petioles that are 1.5-2.5 cm long. The leaves have gland above and rachis which is 4-12 cm long with numerous raised glands all along its upper side (Ramachandran, 1993). The pinnae occur in 8-30 pairs, pinnules in 16-70 pairs (Costermans, 1994). *A. mearnsii* is andromonoecious (Moncur *et al.* 1988). Flower heads contain both staminate and bisexual flowers, and thus not all flowers have the ability to produce seed. The proportion of male flowers may be determined by environmental factors during the development of the flowers (Moncur 1988 pers. comm.). The tree has dark brown pods that are finely hairy (Henderson, 1998). Apart from producing copious numbers of seeds the plant generate numerous suckers resulting in monotypic thickets (Nyoka, 2003). The form of the species varies, not only with genotype and damage sustained during growth, but also in response to stand density and soil moisture availability.

They are used for conservation and improvement of soil fertility through nitrogen fixation. Ethnomedicinally it has long been used for the treatment of skin, sexual, stomach and tooth problems. They are used for fuel wood, timber and shelter (Palmberg, 1981). These species can provide the nutrients and therapeutic ingredients to prevent, alleviate or treat many diseases in humans (Maslin and Stirton, 1997). *Acacia mearnsii* bark extract, an important renewable industrial raw material, has traditionally been used in leather tanning and adhesive processing (Roux *et al*., 1975 and Nursten, 1999) and is now recognized to have strong physiological effects, such as antioxidant, anti-inflammatory, and enzyme-inhibitory activities (Kusano *et al*., 2011; Xiong *et al.*, 2017and Makino *et al.,* 2011)

Acacia contain a variety of bioactive components such as flavonoids, alkaloids, tannins and phenolic acids. The most prominent substances in many Acacia species are complex phenolic compounds (condensed tannins) and polysaccharides or gums (Maslin and Stirton, 1997). These compounds are responsible for numerous biological and pharmacological properties of *Acacia* due to their strong antioxidant and free radical scavenging activities (Chopra *et al.,* 1999). The pharmacological activity of *Acacia* species stated that the plants have acacatechin, cate- chutannic acid and quercetin compounds (Wyk *et al*., 2000). The bark of *A. mearnsii* is known to contain about 20-40% tannins and 70% proanthocyanidins (Young *et al*., 1986). Study of total flavonoid, total phenolic contents and its correlation with antioxidant activity of the extracts was studied by Olajuyigbe and Afolayan, (2011). In addition, *A. mearnsii* bark extract has an antidiabetic effect in vitro (Xiong *et al*., 2017). Yazaki (2015) carried out studies in the barks of *Acacia mearnsii* De

Wild. (black wattle) and he found that it contains significant amounts of water-soluble components, known as “wattle tannin”, which has been used for producing tanned leather for more than one hundred years and for the manufacture of water-resistant and structural wood adhesives for more than fifty years.

Helichrysum bracteatum

Synonyms

Xerochrysum bracteatum.(Vent) Tzvelev;

Bracteantha bracteata (Vent) Anderb & Haegi;

Helichrysum lucidum Henckel and *Helichrysum*

chrysanthum, Pers

The genus *Helichrysum* has attractive yellow flowers displayed by several species (Pooley, 2003) belongs to the family Asteraceae, tribe Inuleae and subtribe Gnaphaliinae (Hilliard, 1983). It is the largest family of flowering plants in the world. The family includes over 1,600 genera and 23,000 individual species. Many members of the Asteraceae family are important for medicinal, ornamental, and economic purposes (Gao *et al*, 2010).

The genus *Helichrysum* consists of approximately 500 species and although *Helichrysum* species are also found in southern Europe, south-west Asia, southern India, Sri Lanka (previously Ceylon), and Australia, most species occur in Africa, including Madagascar (Hilliard, 1983) and higher occurrence in Mediterranean areas of Europe (Facino *et al*., 1988; Perrini *et al*.,2009 and Fortunato *et al*., 2010). It is estimated that amoung 500 species, 20 species are found in Turkey (Fujita *et al*., 1995 and Sezik *et al*., 1991, 2001). In South Africa (including Namibia), 244-250 species are widely distributed based on their morphological diversity 30 morphological groups have been identified using the shape and size of the flower heads as differentiating characteristics (Hilliard, 1983). Several of these species are known by the same vernacular names, for example *H. cymosum, H. nudifolium,*
H. odoratissimum (Wyk *et al.,* 2000).

The name *Helichrysum* is derived from the Greek words ‘Helios’ and ‘chryos’, which means, ‘sun’ and ‘gold’, in direct relation to the fact that theplant species of this genus

typically have inflorescences of a bright yellow colour (Perrini *et al*., 2009).The genus

Helichrysum compresses a large number of species in South Africa that is used traditionally, which is associated with infections. It is considered a very complex genus, with great similarities between some species (Sala, 2001). A number of compounds isolated from the South African *Helichrysum* species to check it's antimicrobial and other activities. Most of the phytochemical research was done in the 1970's and 1980's by the group of Bohlmann and often no connection was established between compounds isolated and any biological activity.

Helichrysum is found to be annuals, herbaceous perennials or shrubs growing to be height of 90cm (Mathekga, 2001 and Lourens *et al*., 2004). It is commonly called strawflower. It may be yellow, orange, pink, deep rose, red, wine, magento, purplor white in colour. These flowers bloom from summer to early autumn. Members of the genus *Helichrysum* are usually aromatic, perennial shrubs, having dense leaves with hardy flower heads. Their flowers come in an array of almost all colours, except blue. There are many capitula and generally flat-topped corymbs or panicles. The corolla lobes show glandular hairs at the abaxial surface (Davis *et al*., 1988 and Guner *et al*.,2000)..*Helichrysum bracteatum* is usually an erect annual herb, 20–80 cm high, simple or rarely branched at base; stems are scabrous-hairy and minutely glandular hairs. Leaves are oblanceolate or narrow- elliptic to lanceolate, mostly 1.5–10 cm long, 5–20 mm wide, scabrous-pubescent, especially on margins. Heads terminal on all branchlets, forming irregular leafy inflorescences, usually on peduncles to 15 cm long; heads 1–2 cm long, 2.5–3 cm diam (Brown and Bayer, 2001). Flowers comprised of many tubular flowers and scarious bracts (Everett, 1980). The scarious bracts are large and coloured like a corolla. They maintain their aesthetic value without wilting or discoloration for many years, even after cutting. *Helichrysum bracteatum* is, therefore, suitable as a dried flower. These plants have the scarious bracts or sepals and their flowers are large and coloured like a corolla. The water content of the scarious bracts of *H. bracteatum* 'Jumbo Yellow'. Thus, scarious tissues have a low water content, while growing plant tissues typically contain 80 to 90 % water. Wood is composed mostly of dead cells, and has a low water content. For instance, the sapwood that functions in transport in via the xylem contains 35–75 % water (Taiz and Zeiger, 2002). The flower heads occur as either solitary or in compact or spreading inflorescences. The aerial parts are usually hairy or woolly and plants occur as herbs or shrublets that are sometimes dwarfed and cushion forming. They are also often aromatic (Pooley, 1998; Pooley, 2003; Van Wyk *et al.,* 2000). The scarious bracts are large and coloured like a corolla. They maintain their aesthetic value without wilting or discoloration for many years, even after cutting. *Helichrysum*

bracteatum is, therefore, suitable as a dried flower. It is called an 'eternal flower' and due its coloured, scarious bracts which maintains their aesthetic value without wilting or discoloration for many years (Nishikawa *et al*., 2008).

Various studies on the genus *Helichrysum* have demonstrated to have antimicrobial and antiseptic properties in many species (Ramanoelina *et al*., 1987; Medici *et al*., 1992 and Afolayan and Meyer, 1997). Theophrastus reported that the '*Helichrysum*' plants are used in the treatment of burns (mixed with honey) and stings/bites of venomous animals (Scarborough, 1978). Decotion prepared from various species have been widely used as a folk remedy in Turkish folk medicine as diuretic, lithagogue and for stomechache (Fujita *et al*., 1995; Sezik *et al*., 1991, 2001). Turkish species are widely used as di- uretics, as lithagogues, for stomachache, for anti-asthmatic properties, against kidney stones, and as herbal tea in Turk- ish folk medicine (Sezik *et al*., 1991 and Sezik *et al*., 2001). The capitulums of Helichrysum species are also used to decrease blood glucose level in diabetes mellitus in Turkey (Baser *et al.,* 1986). They are normally used as herbal infusions and are associated with numerous biological activities such as antioxidant, antimicrobial, anti- inflammatory, anti-allergic, in addition to relief of abdominal pain, heart burn, cough, cold and wounds (Carini *et al*., 2001 and Matic *et al*., 2003).

Several Helichrysums are widely used in Southern African as traditional medicine .Helichrysum species was used to treat nervousness and hysteria was first recorded in 1727 by Boerhaave (Professor of Botany and Medicine at the University of Leiden from 1701 to 1738) (Scott and Hewett, 2008). In European medicine, flower heads from *Helichrysum arenarium* (Helichrysi flos; synon. Flores Stoechados citrinae or Flores *Gnaphalii arenarii*) are used to treat peptic discomfort and are also used as an additive to tea mixtures, mainly to improve their appearance. This diuretic tea has value in the supportive treatment of cholecystitis and spastic disorders of the gall bladder and urinary tract (Van Wyk *et al.,* 2000). Other species such as *H. angustifolium, H. italicum and H. stoechas* are well known in folk medicine and are used for their anti-inflammatory and anti-allergenic properties (Carini *et al*., 2001). The plants are used in different ways, including the preparation of teas, inhalation of smoke and vapours, and placement of leaves in the form of a poultice on wounds to prevent infection (Van Wyk *et al.,* 2000).

There are several recurring South African traditional uses for plants from this genus. They are often used to treat respiratory conditions and leaves are often applied as wound

dressings. They are used in the treatment of gastro-intestinal disorders such as abdominal pain and colic and also eye conditions. They also seem to have an effect on the relief of pain and inflammation as they are used to treat menstrual pain, rheumatism, and headaches. The plants are used to fumigate huts and also used as bedding to repel insects (Hilliard, 1983).

The genus of *Helichrysum* is complex, with a wide variety of chemical classes, among which are flavon- oids, chalcones, phloroglucinol derivatives, essential oils, α-pyrones, and diterpenes (Lourens *et al*., 2008). Different compounds like phenolics e.g. flavonoids and chalcones, phthalides α-pyron derivatives, terpenoids, essential oils volatiles and fatty acids have been found in the genus(Czinner *et al*., 2001).

Biological activities of extracts from Helichrysum species have been widely reported (Cosar and Cubucku, 1990; Rios *et al*., 1991; Barberan *et al*., 1988; Aiyegoro *et al*., 2008 and Aiyegoro *et al*., 2009). The compounds responsible for these activities have been identified and isolated from the *Helichrysum* genus is quite broad, covering nearly all the known fundamental classes, with the exception of alkaloids (Tomas-Barberan *et al*., 1988; Rios *et al*., 1991 and Afolayan and Meyer, 1997).

Tuan and his co-workers worked on photoperiodism and reported that the floral initiation and flower development is affected by temperature and photoperiod and they concluded that photoperiod is most important than the temperature (Tuan *et al.*, 2013). They recorded that various irrigation interval treatments has affected total soluble sugars content and amounts of total soluble phenols were significantly increased by increasing irrigation intervals.

Grevillea robusta

Synonyms

Grevillea venusta A. Cunn. ex Meisn.,

Stylurus robustus A. Cunn. O. Deg.;

Grevillea belongs to the family Proteaceae and it is the largest genus in this family. The family includes about 54 genera and over 1,000 species. The members consist of shrubs and trees, very rarely herbs, usually with spirally arranged, more or less leathery leaves, subdivided or entire, without stipules. The flowers are otten clustered inlarge spikes or heads;

the fruit is a dehiscent capsule or follicle, or an indehiscent stone fruit or nut (Ewart, 1930). Few plants have economic value. Most members of the family are markedly xerophytic.

Grevillea is a large genus of about 170 species of trees and shrubs distributed throughout Australia. It is native to Australia with high commercial value as landscape ornamentals and cut flowers (Joyce and Beal, 1999). *Grevillea robusta*, commonly known as the southern silky oak or silky oak, or Australian silver oak planted as ornamental in many warm temperate and semi-tropical climates. It is not closely related to the true oaks, *Quercus*. It is a native of eastern coastal Australia, although some species are also native to Indonesia, Papua New Guinea and the South Pacific region (Cock, 2012) in riverine, subtropical and dry rainforest environments receiving more than 1,000 mm per year of average rainfall. *Grevillea robusta*. A. Cunn.exr.Br is also known as savuku in Tamil (Matthew, 1995).

G. robusta is distributed in the tropical highlands of India. It has been planted extensively as shade for tea and coffee (Fenton *et al*., 1997). It is the largest species in the genus *Grevillea*. It has no recognized subspecies or varieties, and no hybrids with other species have been recorded (McGillivray, 1993). *Grevillea* contains 362 species, out of which 357 are found in Australia (Makinson, 2000). It is frequently used as a wind break although opinions differ as to its wind firmness and branch shedding tendencies (Jackson *et al*., 2000). Silk oak is an important honey tree in India where it is also regarded as a good fuel wood producer (Jackson *et al*., 2000). The tree produces an attractively figured easily worked wood, which was once a leading face veneer in world trade, where it was marketed as "lacewood". The wood contains an allergen that causes dermatitis for many people (Ahmed *et al*., 2014).

In Australia, *G. robusta* is a relatively large evergreen tree that is 20–30 m tall but it seems to be smaller in other non native areas (Little and Skolmen, 2003). The crown is conical and symmetrical with major branches spaced at intervals of about 1 m and projecting upwards at an angle of 45º. Bark on the trunk is dark gray and furrowed into a lace-like pattern. The fern-like foliage of this species is very distinctive. Its leaves are deeply dissected which are 10-34cm long and 9-15cm wide, variably pinnate to bipinnate, and subdivided which gives fern-like appearance with the complete flower head, or inflorescences, being approximately 12–16 cm long with numerous yellow-orange flowers (Australian Government, 2008) in a bottlebrush-like formation. It is native to eastern Australia, but is now widely planted in many countries, mainly as an ornamental and shade tree, with for

example, over 2 million trees having been planted in Hawaii (Australian Government, 2008; Little and Skolmen, 2003; Cook and Freeman, 1997; Motooka *et al*., 2003). Petioles are 1.5-6.5cm long. The bright orange flowers, about 2 cm in length, are borne in numerous pairs along the flower spikes, on pedicels 1.5 cm long. Fruits are two-seeded follicles 2 cm in length, with a slender persistent style. Seeds are winged, 13–19 mm long x 8-10 mm wide and 0.8–0.9 mm thick, with a papery wing around the brown, ovate central seed body. silver oak is fast growing species, its growth in India is not as fast as in Australia (Umashankar *et al.*, 2012). It is native to eastern Australia, but is now widely planted in many countries, mainly as an ornamental and shade tree, with for example, over 2 million trees having been planted in Hawaii (Australian Government, 2008; Little and Skolmen, 2003; Cook and Freeman, 1997; Motooka *et al*., 2003). Its pale pinkish-brown wood is attractive due to the prominent rays, which resemble oak; wood from *G. robusta* is also used for flooring, furniture and telegraph poles (Cook and Freeman, 1997 and Little and Skolmen, 2003). Its seeds are wind-dispersed, *G. robusta* is naturalized and has become a noxious invasive species (Little and Skolmen, 2003; Cook and Freeman, 1997; Motooka *et al*., 2003 and (Invasive Species Specialist Group, 2008). It is claimed that the leaves of *G. robusta* produce an allelopathic substance that inhibits the establishment and development of other species (Invasive Species Specialist Group, 2008).

Recent research have found few effective bioactive compounds from *Grevillea robusta* namely 4- hydroxyacetophenone, Bisnorstriatol, Grasshopper ketone, P-coumaric acid, which may be potential inhibitors of estrogen receptor alpha (ER-α) for searching a drug against the breast cancer (Syed Mohammed *et al*., 2016). Chuang *et al*, (2011) worked on the MeOH extract of the leaves from *G. robusta* which showed significant cytotoxicity against human breast carcinoma (MCF-7), lung carcinoma (NCI-H460), and central nervous system carcinoma (SF-268) cell lines. Fractionation of the extract was found to consist of three new compounds, graviquinone, cis-3-hydroxy-5-pentadecylcyclohexanone, and methyl 5-ethoxy-2-hydroxycinnamate. Several phenolic glucosides, cytotoxic 5- alkylresorcinol metabolites, 5-alkylresorcinol glucosides derivatives have been isolated from *G. robusta* (Ahmed *et al*., 2000; Chuang and Wu, 2007; Yamashita *et al*., 2008 and Yamashita *et al*., 2010). Arbutin derivatives and resorcinol derivatives are widely present in *G.robusta* (Ahmed *et al*., 2000). The methanol extract of its timber exhibit potent leishmanicidal activity (Takahashi *et al*., 2004). The total phenolics and extractable condensed tannins in leaf stem bark and fine root of *G. robusta* was determined by

Shu-Dong and his co-workers. They also evaluated the ability of these PA extracts to scavenge 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radicals (Shu-Dong *et al.*, 2012). The flowers and fruits of *G. robusta* contain toxic hydrogen cyanide (Everett, 1980). Presence of the chemical substance tridecylresorcinol is responsible for contact dermatitis (Menz *et al.*, 1986).

G. robusta is rich in proanthocyanidins (PAs), but to the best of our knowledge the information regarding its concentration, structural composition and biological activity is limited. The unexplored PAs from this plant may be potential resources for novel bioactive compounds. PAs also termed as condensed tannins, are a structurally complex subclass of polyphenolic compounds that are widely distributed in the plant kingdom (Crozier *et al.*, 2009).

The effective bioactive compounds from *Grevillea robusta* namely 4-hydroxyacetophenone, Bisnorstriatol, Grasshopper ketone, P-coumaric acid, which may be potential inhibitors of estrogen receptor alpha (ER-α) for searching a drug against the breast cancer (Syed Mohammed *et al*., 2016). Arbutin derivatives and resorcinol derivatives are widely present in *G. robusta* (Ahmed *et al*., 2000).

Previous phytochemical work on *G. robusta* and *G. striata* revealed that most of the compounds isolated have been identified as resorcinol derivatives (Ritchie *et al*., 1965; Ridley *et al*., 1970; Varma *et al*., 1976; Roufogalis, 1999 and Ahmed *et al*., 2000). Some species of *Grevillea* have been reported to cause allergic contact dermatitis, which may be due to the presence of alkylresorcinol in the plant (Menz *et al*., 1986). A methanol extract of *G. robusta* showed potent leishmanicidal activity (Takahashi *et al*., 2004). Some resorcinols isolated from *Grevillea* were evaluated for inhibitory Ca^{2+}-ATPase activity (Roufogalis *et al.*, 1999).

G. robusta possesses significant cytotoxic, thrombolytic and membrane stabilizing activities. The plant is a good candidate for further systematic, chemical and biological studies to isolate the active principles (Sharif Ullah *et al*., 2014). Also Cytotoxic 5- Alkylresorcinol Metabolites from the Leaves of *Grevillea robusta* were reported by Hsien and Pei-Lin Wu (2007).

Passiflora tarminiana

Synonyms

Passiflora mixta L.f

Passiflora tomentosa

Passiflora tripartita Holm-Nie., Jorg. & Law.

Passiflora mollisima Kunth

Tacsonia mollissima Kunth

The genus *Passiflora* belongs to the family Passifloraceae or Passion Vine family. It is a relatively small family with approximately 36 genera and about 930 species. The genus contains 450 species classified into 12 subgenera. Most of the genus producing edible fruit belongs to subgenera *Passiflora* and *Tacsonia*. Among this more than 47 species distributed in the Andean mountains and is classified into ten sections, five of which are monospecific (Escobar 1988). The subgenus Tacsonia includes 7 cultivated *Passiflora* and Tacsonia are closely related to each other (Fajardo *et al.,* 1998, Sanchez *et al*., 1999). The genus *Passiflora* has highly economic importance (Pereira *et al.,* 2015). The *Passiflora* species were discovered by Spanish missionaries in South America (Ozaroski and Thiem, 2013).

The *Passiflora* species are widely used as a traditional medicine especially as sedatives and anxiolytics and also used in pharmacological and cosmetics industries. Some of the species like *Passiflora edulis.* Sims and *Passiflora laurifolia*. L are widely cultivated for their edible fruits. And some species like *Passiflora morifolia* mast, *Passiflora suberosa litoralis.* Kunth are cultivated as ornamental plants because of their unique and spectacular flowers (silva *et al*., 2014; Calapai *et al*., 2013). Majority of the species are found in tropical regions of India, China, Australia and Pacifia Islands (silva *et al.*,2014 and Cutri *et al*., 2013). Their fruits have intense, delightful and peculiar aroma and flavour. So the early Christian missionaries of South America described it as the symbol of the passion of Christ (Miroddi *et al*., 2013). In the

past years Brazil has emerged as the world's largest producer of passion fruit almost 60% of the global production (Oliveira *et al.*, 2016).

P.tarminiana, also known as maypop, apricot vine, passion vine, and granadilla is a woody wine from the Andean highlands (2000–3600 m) of South America, has successfully invaded in the upper sholas of the Nilgiris, often climbing to heights of 30 ft (10 m) tall, with a thick, woody stem (Montanher, 2007 and Beninca, 2007). They are self pollinated (Myers *et al*., 2000). It is also called by banana passion fruit.these are woody climbers called Lianas, Leaf with blades 6-16 cm long, 7-20 cm wide, deeply 3-lobed. Flower is pendent, salverform, 6-9 cm in diameter, peduncles solitary, 3.8-10 cm long. Fruit is yellow at maturity, pericarp softly coriaceous, obovate to oblong, 6-8 cm long, pubescent, aril orange (Wagner *et al*., 1999) which is sometimes sweet and other times sour with a typical fine and special aroma (Duarte and Paull, 2015).

P.tarminiana is used as a food crop in South America and in some places it is cultivated at homes for its fruit. Fruit is considered a rich source of vitamin C (40 mg per 100 g of edible fruit), which confers it good antioxidant capacity (Simirgiotis *et al.*, 2013). The plant is not a forest pest because native insects intensively feed on it (Gilbert, 1975). The vine is capable of climbing 20 m into the crowns of older overstorey trees, forming a curtain to the forest floor (Rosa, 1984).

Passion flower is generally considered to be a safe herb with few reported serious side effects. In cases of side effects, the products being used have rarely been tested for contamination, which may have been the cause. Cyanide poisoning has been associated with *Passiflora* fruit, but this has not been proven in human studies. Rapid heart rhythm, nausea, and vomiting have been reported. Side effects may also include drowsiness /sedation and mental slowness. Patients should be cautious when driving or operating heavy machinery. Passion flower may theoretically increase the risk of bleeding and affect blood tests that measure blood clotting (Kapadia *et al*., 2002). Passion flower has a long history of use for symptoms of restlessness, anxiety, and agitation (Dhawan *et al.*, 2003).

Even though it is used as a medicine for various purposes, it has its own demerits too. The traditional uses includes alcohol withdrawal, antibacterial, anti-seizure, anti-spasm, aphrodisiac, asthma, attention deficit hyperactivity disorder (ADHD), burns (skin), cancer, chronic pain, cough, drug addiction, Epstein-Barr virus, fungal infections, gastrointestinal discomfort (nervous stomach), Helicobacter pylori infection, hemorrhoids, high blood pressure, menopausal symptoms (hot flashes), nerve pain, pain (general), skin inflammation, tension and wrinkle prevention (Dhawan *et al*., 2002). Several species of *Passiflora* have

been employed widely as a folk medicine because of its sedative and tranquillizer activities (Barbosa *et al*., 2008).

Phytochemicals present in them are responsible for their medicinal properties which are listed below. Most of the species contains beta- carboline harmala alkaloids. The majority of the active components in this plant are C-glycosyl flavones based on apigenin and luteolin, while harman alkaloids are found in trace amounts (Hiremath *et al*., 2000; Rehwald *et al*., 1994 and Raffaelli *et al*., 1997). The *Passiflora* family contains small amounts of harmala alkaloids, harmane (passaflorine), and possibly harmine (telepathine), harmaline, harmol, and harmalol (Lawrence, 1989) Many different types of glycosides are present in passion flower such as apigenin, homoorientin, 7-isoorientin, isoshaftoside, isovitexin, kaempferol, lucenin, luteolin, n- orientin, passiflorine (named after the genus), quercetin, rutin, saponaretin, saponarin, shaftoside, vicenin and vitexin. In some cases this glycoside occurs with simple -D-glucopyranosides: tetraphyllin A, deidaclin, tetraphyllin B, volkenin, epivolkenin and taraktophyllin. (Jaroszewski and olafsdottir, 2002). Some other glycosides present in *Passiflora* are the hydrocarbon nonacosane and the anthocyanidin pelargonidin-3- diglycoside (Duke, 2008).

Orientin is a flavone, a chemical flavonoid-like compound found in the passion flower. Isoorientin (or homoorientin) is the luteolin-6-C- glucoside which is isolated from the passion flower (Linda *et al*., 2006). Passion flower contains many alkaloids, flavonoids as well as many organic compounds such as organic acids. This genus is rich in formic, butyric, linoleic, linolenic, malic, myristic, oleic and palmitic acids as well as phenolic compounds, and the amino acid -alanine. Some species contain ester such as ethyl butyrate, ethyl caproate, n-hexyl butyrate and n-hexyl caproate which give the fruits their flavor and appetizing smell. Sugars, contained mainly in the fruit, are mostly d-fructose, d- glucose and raffinose. Among enzymes, *Passiflora* was found to be rich in catalase, pectin methyl esterase and phenolase . Most of the species of *Passiflora* contain the saponins. Saponins are common constituents of plants that exhibit a broad spectrum of biological activities (Birner and Nicolls, 1973; Perry *et al*., 1991) and frequently possess hemolytic, cytolytic and bactericidal activities (Rao and Song, 1995; Li *et al*., 2005). also plasma cholesterol-lowering activity (Chandel and Rastogi, 1980) . However, not all *Passiflora* species contain saponins in their leaves (Akiyama *et al*., 1980).

The local healers use the leaves of *P. tarminiana* as an antidiabetic drug . Herbal extracts of *Passiflora* sp also used in traditional medicines to cure many diseases like diarrhea, intestinal tract, throat, ear infections, fever and skin diseases and in Ayurveda for several remedies such as sedative, anxiety and hypertension. The plant is used as antifungal, antibacterial and antifeedant (Edwin *et al*., 2007). Alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds are known in the genus (Patel *et al*.,2011). The plant is used commonly by the local traditional practitioners of Ooty to treat diabetes is *Passiflora tarminiana* commonly known as Banana Passion fruit. *P. mollisima* is used as anti feedant, anti fungal, and antibacterial (Edwin *et al*., 2005). The reported main constituents of *P. tarminiana* are alkaloids, saponins, ß avonoids, triterpenoids, and proteins (Rerup, 1970).

Materials & Method

MATERIALS AND METHODS

3.1 Collection of plant materials

Plant materials were collected at respective places as given in the table.1. Collected plants were authenticated by Dr. Stephen, Assistant Professor, American College, Tamil nadu, India.

Table 1. List of plant materials taken for this study

S.No	Plant Name	Common Name	Family	Place
1	*Acacia mearnsii* De Wild.	Black Wattle	Fabaceae	Kovilmedu, Ooty
2	*Bracteantha bracteata* Vent	Dragan Hill monarch	Asteraceae	Kovilmedu, Ooty
3	*Grevillea robusta* A.Cunn	Silver Oak Tree	Proteaceae	Kovilmedu, Ooty
4	*Passiflora tarminiana* Kunth	Bannana Passion fruit	Passifloraceae	Pudhumund, Ooty

Leaves and stems were rinsed with distilled water. Afterwards, the samples were dried under shade, ground and sieved for extraction process.

3.2 Solvent extraction (Farombi *et al.*, 2003)

3.2.1 Aqueous extraction

Powdered samples (10g each) was weighed and soaked separately in 50ml cold water in a conical flask stoppered with rubber cork and kept in orbital shaker for 24 hours. It was then filtered off using sterile filter paper (What Man No: 1) into a sterile conical flask.

3.2.2 Methanol Extract - Maceration

Powdered samples (10 g each) were weighed. The extracts were prepared in same as in aqueous extraction and stored at 4º C in air tight containers.

3.2.3 Ethyl Acetate Extract - Maceration

Powdered samples (10 g each) were weighed. The extracts were prepared in same manner as in aqueous extraction and stored at 4º C in air tight containers.

Plate 1: Acacia mearnsii De Wild

Kingdom - Plantae
Division - Magnoliophyta
Class - Magnoliopsida
Order - Fabales
Family - Fabaceae
Genus - *Acacia*
Species - *mearnsii*

Plate 2: Bracteantha bracteata Vent

Kingdom - Plantae
Division - Magnoliophyta
Class - Magnoliopsida
Order - Asterales
Family - Asteraceae
Genus - *Bracteantha*
Species - *bracteata*

Plate 3: Grevillea robusta A.Cunn

Kingdom - Plantae
Division - Magnoliophyta
Class - Magnoliopsida
Order - Laurales
Family - Lauraceae
Genus - *Grevillea*
Species - *robusta*

Plate 4: Passiflora tarminiana Kunth

Kingdom - Plantae
Division - Magnoliophyta
Class - Magnoliopsida
Order - Violales
Family - Passifloraceae
Genus - *Passiflora*
Species - *tarminiana*

Plate 5. Acacia mearnsii powder and its extracts

Leaf Powder

Ethyl Acetate Extract

Methanol Extract

Aqueous Extract

Stem Powder

Ethyl Acetate Extract

Methanol Extract

Aqueous Extract

Plate 6. Bracteantha bracteata powder and its extracts

Leaf Powder

Ethyl Acetate Extract

Methanol Extract

Aqueous Extract

Stem Powder

Ethyl Acetate Extract

Methanol Extract

Aqueous Extract

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Plate 7. Grevillea robusta powder and its extracts

Ethyl Acetate Extract

Methanol Extract

Leaf Powder

Aqueous Extract

Ethyl Acetate Extract

Stem Powder

Methanol Extract

Aqueous Extract

Plate 8. Passiflora tarminiana powder and its extracts

Ethyl Acetate Extract

Methanol Extract

Leaf Powder

Aqueous Extract

Ethyl Acetate Extract

Stem Powder

Methanol Extract

Aqueous Extract

3.3 Physiochemical parameters

3.3.1 Determination of moisture content (AOAC, 1990)

A quantity of 5 g of sample was dried in the oven at 105˚C and moisture content was calculated as percentage.

$$\text{Moisture Content} = \frac{W - D}{W} \times 100$$

Where,

W = Wet weight

D = Dry weight

3.3.2 Determination of solubility percentage (Kokate, 1994)

Procedure

- ✓ About 5 g of powdered material was weighed and macerated with 100 ml of methanol in a closed flask for 24 hours shaking frequently during the first 6 hours and kept undisturbed for 18 hours.
- ✓ Thereafter, it was filtered rapidly taking precautions against loss of the solvent.
- ✓ About 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed, swallowed dish dried at 105˚ C for 6 hrs and cooled in a desiccator and weighed.
- ✓ The content of extractable matter (% w/w) air-dried material was calculated as follows.

$$\text{soluble extractives (\% w/w)} = \frac{\text{(Weight of residue) x 5 x 100}}{\text{Weight of the sample}}$$

3.4 Phytochemical Screenings

The extracts obtained in the successive extraction process from leaves and stems of four plants were subjected to various preliminary phytochemical screening for the identification of phytoconstituents present.

3.4.1 Test for Carbohydrates

Molisch's test:

To a small amount of the extract few drops of Molisch's reagent was added followed by the addition of conc. H_2SO_4 along the sides of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 ml of distilled water. Formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates.

3.4.2 Test for Reducing Sugar

Benedict's Test:

Extract (2 ml) were treated with 2 ml of Benedict's reagent and heated in a water bath for 3 minutes. Presence of green, red or yellow ppt indicates the presence of reducing sugar.

3.4.3 Test for Proteins

Millon's test:

Extract (3 ml) was mixed with 5 ml of millon's reagent. White precipitate is formed. On warming precipitate turn's brick red or the precipitate dissolves giving red colored solution.

Biuret test:

To extract (3 ml) few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple colour. On addition of alkali, it becomes dark violet.

Tannic acid test:

To Extract (2ml) few drops of 10% tannic acid was added for the formation of white precipitate.

Ninhydrin test:

Extract (2 ml) was mixed with 3-4 drops of Ninhydrin's reagent and then heat. A intense Blue colour is formed which indicates the presence of proteins.

Test for Xanthoprotein:

To 2 ml of extract, a few drops of concentrate nitric acid and 1 ml of ammonia were added. Appearance of a red precipitate indicates the presence of xanthoprotein.

3.4.4 Vitamin C

DNPH test:

Sample was treated with Dinitrophenyl hydrazine and sulphuric acid. Formation of yellow precipitate indicates the presence of vitamin C**.**

3.4.5 Test for Alkaloids

Mayer's test:

Sample (2 ml) was treated with few drops of Mayer's reagent. Appearance of white precipitate indicated the presence of alkaloids.

Wagner's test:

Sample (2 ml) was mixed with few drops of Wagner's reagent. Appearance of reddish brown precipitate indicated the presence of alkaloids.

Hager's test:

Sample solution and few drops of Hager's reagent were added. Appearance of yellow precipitate indicated the presence of alkaloids.

3.4.6 Tests for Flavonoids

Flavones -Shinoda test:

Sample extract was treated with 5 ml of 95% ethanol, few drops of concentrated Hydrochloric acid and 0.5 g of magnesium turnings were also added. Pink colour was observed. Addition of increasing amount of sodium hydroxide to the residue shown yellow coloration, this decolorized after addition of acid indicates the presence of flavones.

Flavanones:

Sample extract (1 ml) was taken and 10 % of sodium hydroxide was added. Yellow to orange colour formation indicates the presence of flavanones.

Sample extract (1 ml) was taken and few drops of con.H_2SO_4 was added. Orange to crimson colour formation indicates the presence of flavanones

Alkaline test:

Sample extract (1 ml) was treated with few drops of sodium hydroxide. Yellow colour is formed which turns to be colourless after adding a few drops of diluted acid.

3.4.7 Test for Glycosides:

Legal's test

Sample extract was mixed with few drops of pyridine and 2 drops of 2 % sodium nitroprusside was added. To the reaction mixture 0.5 ml of 20 % sodium hydroxide was added. Appearance of pink to red color indicated the presence of glycosides.

Bromine water test:

Sample (1 ml) was treated with 3 drops of bromine water and the formation of yellow precipitate indicates the presence of glycosides

3.4.8 Test for cardiac Glycosides

Keller-killani test:

Five ml of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under layered with 1 ml of conc. sulphuric acid, a brown ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

3.4.9 Test for anthroquinone

Borntragor's test

Extract (0.5 ml) was added with 5-10 ml of dilute hydrochloric acid and boiled on water bath for 10 minutes. Solution was filtered and filtrate was extracted with benzene and mixed with ammonia solution. Red color was obtained in ammonia layer that indicated the presence of anthraquinone glycosides.

3.4.10 Test for Terpenoids

Salkowski's test:

1 ml of the extract was mixed with 0.5 ml of chloroform ($CHCl_3$) and concentrated 6M H_2SO_4 (3ml) was carefully added forming a layer. A reddish brown coloration of the interface indicated the presence of terpenoids..

3.4.11 Test for Diterpenes:

Extracts (3 ml) are treated with a few drops of copper acetate solution. Formation of emerald green colour indicates presence of diterpenes.

3.4.12 Test for Triterpenoids

Extract (5 ml) was dissolved in chloroform (2 mL) and then acetic anhydride (1 mL) was added to it. Concentrated sulphuric acid (1 mL) was added to the solution. Formation of reddish violet colour shows the presence of triterpenoids.

3.4.13 Test for Saponin

Foam test:

To 1 ml of the extract 5 ml distilled water was added and shaken vigorously. Formation of foam indicated presence of saponins.

3.4.14 Test for phenols

Ferric chloride test:

Extract (2 ml) was treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour. To 1 ml of the extract, 2 ml of distilled water, 3 drops of 10% aqueous ferric chloride ($FeCl_3$) and 3 drops of potassium Ferro cyanide were added. Formation of blue or green color showed the presence of polyphenols.

Extract (2 ml) was treated with 3ml of 10 % lead acetate. Formation of precipitate indicates the presence of phenols.

3.4.15 Test for Tannins (Trease and Evans, 1989)

To 1ml of extract solution, 4 ml of water and 1-2 drops of 10 % ferric chloride solution was added. Blue colour indicates gallic tannins and green black catecholic tannins

3.4.16 Test for Phlobatanins:

0.5 g extract was dissolved in distilled water and filtered. The filtrate was boiled with 2M HCl solution. Formation of red precipitate showed the presence of phlobatanins.

3.4.17 Test for Quinones

To 1ml of test solution Alcoholic KOH solution was added separately. Quinones were indicated by colour ranging from red to blue.

3.4.18 Test for Sterols

Extract (2 ml) was treated with 2 ml of trichloroacetic acid. On heating the colour changes from red to violet. This indicates the presence of sterols

3.4.19 Test for Phytosterols

Libermann burchard's test:

Extracts were treated with few drops of acetic acid and 3 ml of acetic anhydride and few drops of con.H_2SO_4 Formation of reddish brown precipitate shows the presence of phytosterols.

3.4.20 Test for Oxalate

2 ml of extract was mixed with few drops of glacial acetic acid for the formation of greenish black colouration.

3.4.21 Test for Anthocyanin

1 ml of extract was treated with 2M Hcl (1 ml) and 1 ml of NH_3 (4M) Blue or violet colour formation indicates the presence of anthocyanin.

3.4.22 Test for Resin

5 ml of distilled water was added to the 3 ml of the methanol extract for turbidity, which indicates the presence of resins in the plant sample.

3.4.23 Test for Catechin

Match stick was dipped in plant extract, dried and then moistened with concentrated HCl. Warm near flame, a red or pink wood is produced which shows the presence of catechin.

3.4.24 Test for steroids

In a dry test tube 2 ml of extract and 10 drops of acetic anhydride was added then 2 to 3 drops of conc. H_2SO_4 was added. A violet colour is formed which changes into blue green colour which indicates the presence of steroids.

3.4.25 test for Emodins:

2 ml of extract was treated with 2 ml of NH_4OH and 3 ml of Benzene. A red colour is formed which indicates the presence of emodins.

Data collected

The change of colour was observed when the test reagent was added to the prepared sample for the phytochemical test. The result was recorded as present (+) or absent (-) depending on the outcome of the test.

3.5 Quantitative test

The biochemical parameters analyzed were

1. Carbohydrates
2. Proteins

3.5.1 Biochemicals parameters

3.5.1.1 Total Carbohydrates: (Hedge and Hofreiter, 1962)

Principle

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone, a green coloured product with an absorption maximum at 630nm.

Materials

- 2.5N HCl
- Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice cold 95 % H_2SO_4 prepared fresh before use.

Standard glucose (Stock): Dissolved 100 mg in 100 ml of water.

Working standard – 10 ml of a stock solution was diluted to 100 ml distilled water. After adding few drops of toluenetored in refrigerator after adding a few drops of toluene.

Procedure

- About 100 mg of the sample was taken in a boiling tube and it was hydrolysed by keeping it in a boiling water bath for three hours with 5ml of 2.5N HCl and cooled to room temperature.
- Then it was neutralized with solid sodium carbonate until the effervescence created.
- The volume was made up to 100 ml and centrifuged.
- The supernatant was collected and 0.5 and 1 ml aliquots were taken for analysis.
- The standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and '0' served as blank.
- The volume was made up to 1 ml in all the tubes including the sample test tubes by adding distilled water.
- Then, 4 ml of anthrone reagent was added and heated for eight minutes in a boiling water bath.
- Then it was cooled rapidly and the green colour developed was read at 630 nm.
- A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.
- From the graph, the amount of carbohydrates present in the sample tube was calculated.

Calculation

Amount of carbohydrates present in 100mg of the sample is calculated by

$$\frac{\text{mg of glucose}}{\text{volume of test sample}} \times 100$$

3.5.1.2 Estimation of protein (Lowry *et al*., 1951)

Principle

The blue colour developed by phosphomolybdic phosphotungstic components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured in the Lowry's method.

Materials:

- Reagent A - 2 % sodium carbonate in 0.1 sodium hydroxide
- Reagent B - 0.5 % copper sulphate ($CuSO_4.5H_2O$) in 1% potassium sodium tartarate.
- Reagent C - Alkaline copper solution : Mixed 50 ml of A and 1 ml of B prior to use.
- Reagent D - Folin-Ciocalteau Reagent.

Stock standard : 50 mg of bovine serum albumin (Fraction V) was weighed and dissolved in distilled water and the volume was made up to 50 ml in a standard flask.

Working standard: About 10ml of the stock solution was diluted to 50 ml with distilled water in a standard flask. One ml of this solution contain 200 µg proteins.

Procedure

Extraction of protein from sample

Extraction is usually carried out with buffers used for the enzyme assay. About 50mg of the weighed sample was ground well with pestle and mortar in 5- 10 ml of the buffered centrifuged. The sample was used for protein estimation.

Estimation of protein

- About 0.2, 0.4, 0.6, 0.8, and 1 ml of working standard were pipette into a series of test tubes and 0.1 ml and 0.2 ml of the sample extract in two other test tubes.

- The volume was made up to 1 ml in all test tubes. A tube with 1 ml of water severed as the blank.
- About 5 ml of reagent C was added to each tube including the blank, mixed well and allowed to stand for 10 minutes.
- Then 0.5 ml of reagent D was added. Mixed well and incubated at room temperature in the dark for 30 min. Blue colour developed was read at 660 nm.
- A standard graph was drawn and the amount of protein present in the sample was calculated.

Calculation

The amount of protein present in the sample was expressed in

$$\text{mg/ g or100g} = \frac{\text{mg of protein}}{\text{volume of the test standard}} \times \text{concentrationof the standard}$$

3.5.1.3 chlorophyll and nitrogen Estimation:

Fresh leaves were taken to read values for chlorophyll content and nitrogen present in all the four leaf samples using Panomex Plant Nutrient Analyser. Triplicate values were taken for further Statistical Analysis.

3.5.2 Phytochemical Parameters

- Phenol
- Polyphenol
- Flavonoid
- Tannin

3.5.2.1 Estimation of Total Phenol content (Malick and Singh, 1980)

Principle

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue coloured complex (molybdenum blue), which can be estimated spectrophotometrically at 650 nm.

Materials

- Ethanol (80 %)
- Folin-ciocalteau reagent
- Na_2CO_3 (20 %)

Stock standard: Gallic acid (100µg/ml in water)

Working standard: Dilute 10 times of stock

Procedure

- Grind 0.5 g of the sample with a pestle and mortar in 10 times volume of 80 % ethanol.
- Centrifuge the homogenate at 10,000 rpm for 20 minutes. Re-extraction is done and the supernatants were pooled and evaporated to dryness.
- Dissolve the residue in a known volume of distilled water (5 ml).
- Pipette out different aliquots (0.2 to 2 ml) into test tubes.
- Make up the volume in each tube to 3 ml with distilled water.
- Add 0.5ml of Folin-Ciocalteau reagent.
- After 3 minutes, add 2 ml of 20 percent Na_2CO_3 solution to each tube.
- Mix thoroughly, place the tube in boiling water for exactly 1 minutes, cool and measure the absorbance at 650nm against a reagent blank.
- Prepare a standard curve using different concentrations of gallic acid.

Calculation

From the standard curve the concentration of phenols in the sample were observed and express as GAE mg of phenols/g of material.

3.5.2.2 Determination of total polyphenol (Malick and Singh, 1980)

Principle

Phenols react with phosphomolybdic acid in Folin - ciocalteau reagent in alkaline medium and produce blue coloured complex (molybdenum blue), which is read in a spectrophotometer at 650nm.

Reagents

- Diluted Folin - Ciocalteau reagent (1:10 dilution)
- 20% Sodium carbonate
- Ethanol

Stock solution: 100 mg of catechol was made up with 100 ml distilled water

Working standard: 10 ml of stock standard was diluted to 100 ml. 1.0 ml of this contains 100 µg of catechol.

Procedure

- ✓ A working standard of 0.5 – 2.5 ml catechol solution corresponding to 50 – 250 µg of catechol were pipetted out into a series of test tubes including samples and standard.
- ✓ The volume was made upto 2.5 ml with water. To all the tubes added 0.5ml of diluted Folin – Ciocalteau reagent.
- ✓ After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added to each tube and mixed thoroughly.
- ✓ The tubes were placed in a boiling water bath for exactly one minute. Cooled and measured at 650nm against a reagent blank.

Calculation

The results were expressed as mg / g tissue.

3.5.2.3 Determination of total flavonoid content (Grubesic *et al*., 2005)

Principle

The content of flavonols was determined by using rutin as a reference compound. This method was based on the formation of complex with maximum absorption at 440 nm.

Reagents:

1. Aluminium chloride solution (20 mg/ml)
2. Sodium acetate (30 mg/ml)

Procedure

- ➢ About 1 ml of each extract was mixed with 1 ml aluminium chloride and 3 ml sodium acetate.
- ➢ After 2.5 hrs the samples were read at 440 nm absorbance.
- ➢ The absorption of standard rutin solution in methanol was measured under the same conditions.
- ➢ A duplicate was carried out for all the determinations.

Calculation:

The amount of flavonoids in plant extracts in rutin equivalents was calculated by the following formula.

$$X = (A - m_0) / (A_0 - m)$$

Where,

X = flavonoid content (μg/ml) in rutin equivalents

A = absorption of plant extraction solution,

A_0 = absorption of standard rutin solution,

m = weight of plant extract (μg)

m_0 = weight of rutin in the solution (μg)

3.5.2.4 Estimation of Tannin (Folin and Ciocalteau Method, 1927)

Principle

The principle behind this method is reduction of Phosphotungstomolybdic acid in alkaline solution to produce coloured complex.

Materials

1. Folin- Ciocalteau reagent
2. Sodium carbonate
3. Tannic acid

Procedure

- To 0.1 ml of the sample extract 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent, 1 ml of 35% sodium carbonate solution were added and diluted to 10 ml with distilled water.
- The mixture was shaken well, kept at room temperature for 30 min and was measured at 725 nm.
- Blank was prepared with water instead of the sample.
- A set of standard solutions of Tannic acid is treated in the same manner as described earlier and read against a blank.

Calculation

The results of Tannin are expressed in terms of Tannic acid in mg/g tissue TAE of extract.

3.6 Antioxidants assays

3.6.1 Enzymatic assays

The enzymatic antioxidant analysed in this present study were Polyphenol oxidase and Peroxidase.

3.6.1.1. Polyphenol Oxidase (PPO) (Esterbauer *et al*., 1977)

Principle

Polyphenol oxidase was copper protein of wide occurrence in nature,which catalase the aerobic oxidation of certain phenolic substrate to quinines, which are auto oxidized to dark brown pigments generally known as melanins. The polyphenol oxidase (PPO) comprises catechol oxidase and laccase.

Reagents

- Tris-HCl (50 mM, pH 7.2).
- Sorbitol (0.4 M).
- Sodium chloride (10 mM).
- Phosphate buffer (0.1 M, pH 6.5).
- Catechol (0.01 M).

Procedure

- ✓ Into a cuvette, 0.2 ml of the sample extract was added to the reaction mixture containing, 2.5 ml of phosphate buffer and 0.3 ml of catechol solution.
- ✓ The change in the absorbance was recorded every 30 sec up to 5 minute.

Calculation

One unit is equal to the changes in absorbance at 495nm/minute

The activity of PPO can be calculated using the formula

Enzyme units in the sample = $K \times (\Delta A/\text{minute})$

where,

K for catechol oxidase = 0.272

K for laccase = 0.242

3.6.1.2. Peroxidase (Reddy *et al*., 1995)

Principle

In the presence of the hydrogen donor pyrogallol or dianisidine, peroxidase converts $H_2O_2$to H_2O and O_2. The oxidation of pyrogallol or dianisidine to a coloured product called purpurogalli can be followed spectrophotometrically at 430nm.

Reagents

- Pyrogallol : 0.05 M in 0.1M phosphate buffer (pH 6.5)
- H_2O_2: 1% in 0.1M phosphate buffer, pH 6.5

Procedure

- ✓ About 3 ml of pyrogallol solution, 0.1ml of the extract was added and the spectrophotometer was adjusted to read zero at 430 nm.
- ✓ To the test cuvette, 0.5ml of H_2O_2 was added and mixed.
- ✓ The change in absorbance was recorded every 30 secondsup to 3 minutes in a spectrophotometer.

Calculation

One unit of peroxidase is defined as the change in absorbance at 430 nm/minute.

3.6.2. Non-enzymatic assays

3.6.2.1. Ascorbic acid (Roe and Keuther, 1943)

Principle

Ascorbate is converted into dehydroascorbate on treatment with activated charcoal, which reacts with 2, 4-dinitrophenyl hydrazine to form osazones. These osazones produce an orange coloured solution when dissolved in sulphuric acid, whose absorbance can be measured spectrophotometrically at 540nm.

Reagents

- TCA (4%)
- 2,4-dinitrophenyl hydrazine reagent (2%) in 9N H2SO4
- Thiourea (10%)
- Sulphuric acid (85%)
- Ascorbic acid (100mg of Ascorbic acid in 100ml of 4% TCA)

Procedure

- ✓ Ascorbic acid solution of 1 mlwere taken and added with 0.1 ml of plant extract.
- ✓ The reaction mixture was made up to 2.0 ml with 4% TCA.

- ✓ To this, 0.5 ml of DNPH reagent was added followed by 2 drops of 10% thiourea solution.
- ✓ The tubes were incubated at 37˚C for 3 h.
- ✓ The osazone formed was dissolved by the addition of 2.5 ml of 85% sulphuric acid.
- ✓ DNPH reagent and thiourea were added to the blank after the addition of sulphuric acid.
- ✓ After cooling the tubes, the absorbance was read spectrophotometrically at 540 nm.

Calculation

The concentration of ascorbate in the sample was calculated and expressed in terms of mg/g tissue.

3.6.2.2 Total polyphenol (Malick and Singh, 1980)

Principle

Phenols react with phosphomolybdic acid in Folin - ciocalteau reagent in alkaline medium and produce blue coloured complex (molybdenum blue), which is read in a spectrophotometer at 650nm.

Reagents

- ➢ Diluted Folin - Ciocalteau reagent (1:10 dilution)
- ➢ 20% Sodium carbonate
- ➢ Ethanol

Stock solution : 100 mg of catechol was made up with 100ml distilled water

Working standard: 10ml of stock standard was diluted to 100ml. 1.0ml of this contains 100µg of catechol.

Procedure

- ✓ A working standard of 0.5 – 2.5ml catechol solution corresponding to 50 – 250µg of catechol were pipetted out into a series of test tubes including samples and standard.
- ✓ The volume was made upto 2.5ml with water. To all the tubes added 0.5ml of diluted Folin – Ciocalteau reagent.
- ✓ After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added to each tube and mixed thoroughly.

- ✓ The tubes were placed in a boiling water bath for exactly one minute. Cooled and measured at 650nm against a reagent blank.

Calculation

The results were expressed as mg / g tissue.

Result & Discussion

Result and Discussion

In this present investigation comparative study in leaf and stem of *A. mearnsii, B. bracteata, G. robusta* and *P. tarminiana* using ethyl acetate, methanol and water as solvents were done and the results obtained were discussed.

4.1 Physiochemical Parameters

4.1.1 Moisture content

The moisture content in leaf was found to be more (3.93 %) in *P. tarminiana* and low (3.15) in *G. robusta*. In stem the moisture content was found to be maximum (3.71 %) and minimum (2.89 %) in *P. tarminiana* and *G. robusta* respectively.

Table: 1 Physiochemical parameters of the four plants

Physiochemical parameters	Moisture content (%)		Solubility (% W/W)					
			Ethyl Acetate		Methanol		Water	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
A. mearnsii	3.85	3.31	3.24	3.38	3.49	3.42	1.89	2.23
B. bracteata	3.22	3.51	3.12	2.46	9.12	2.55	2.22	2.31
G. robusta	3.15	2.89	2.67	3.44	8.26	2.41	3.29	2.25
P. tarminiana	3.93	3.71	4.54	3.89	6.77	2.71	1.91	2.71

4.1.2 Solubility percentage

The extraction of any plant part with a particular solvent yields a solution containing different phytoconstituents. The composition of the phytoconstituents in that particular extract depends upon the nature of plant part and solvent used.

Extractive value among the stem and leaf of different extracts and four different plants were studied and highest value was found in methanol leaf extract of *B. bracteata* (9.12%) and the lowest in aqueous leaf extract of *A. mearnsii*. Within the ethyl acetate extract the highest value (3.89%) and lowest value (2.46) was found in stem of *P.tarminiana* and *B. bracteata* respectively. In the methanol extract *B. bracteata* and *G. robusta* showed

maximum (9.12 %) in leaf and minimum (2.41 %) in stem respectively. Aqueous extract showed solubility percentage of 1.89 % and 3.29 % in *A. mearnisii* leaf and *G. robusta* respectively.

4.2 Qualitative Phytochemical analysis

All extracts of A. *mearnisii, B. bractantha, G. robusta* and *P. tarminiana* were subjected to qualitative chemical test to identify various phytoconstituents present in them.

4.2.1 Phytochemical Screening

All extracts were subjected to test the presence or absence of twenty five compounds and the result obtained were resulted in table 2 and 3. The assays involved here are simple chemical tests that depend on colour or precipitate formation upon interaction with appropriate reagents in test tubes.

When the ethyl acetate leaf extracts were studied in which presence of carbohydrate, alkaloids, flavonoids, glycosoids, phenols, saponin, protein, phytosterols, tannin, oxalate, vitamin C, resin, steroids, reducing sugar and absence of quinines and sterols were observed in all the four plants. While cardiac glycosides was absent in *P. tarminiana*, terpenoids and diterpenes, anthoquinone and terpenoids were absent in *G. robusta* and *P. tarminiana;* diterpenes and anthocyanins were absent in *A. mearnsii* and *B. bracteata* and emodins were absent only in *A. mearnsii.*

When methanolic leaf extract were examined presence of carbohydrates, alkaloids, flavonoids, glycosides, phenol, protein, tannin, quinines, reducing sugar and absence of diterpenes and sterols were found. While few compounds were absent only in any one of the plant they are saponin and terpenoids absent in *B. bracteata*; cardiac glycosides, oxalate and emodin absent in *G. robusta*, steroids absent in *P. tarminiana* and phytosterols absent in both *A. mearnsii* and *G. robusta*.

Aqueous leaf extract showed the presence of carbohydrates, alkaloid, flavonoid, glycosides, phenol, protein, diterpenes, tannins, triterpenoids, vitamin C, catechin, and absence of phlobatannins, sterols, oxalate, anthocyanin and resin. Certain phytoconstituents were absent in one or other plants like saponin absent in *A. mearnsii*; steroids absent in P. *tarminiana*; anthoquinones absent in *A. mearnsii, B. bracteata* and *G. robusta*; phytosterols absent in *A. mearnsii* and *G.robusta.*

Table:2 Qualitative analysis of phytoconstituents in leaf extracts of *A. mearnsii, B. bracteata, G. robusta and P. tarminiana*

S.no	Test		Ethyl Acetate				Methanol				Aqueous			
			1	2	3	4	1	2	3	4	1	2	3	4
1	Carbohydrate (*Molisch's Test)*		+	+	+	+	+	+	+	+	+	+	+	+
2	Alkaloids	*Mayer's*	+	+	+	+	+	+	+	+	+	-	+	+
		Wagner's	+	+	+	+	-	+	+	+	+	+	+	+
		Hager's test	+	+	+	+	+	-	-	-	+	+	+	+
3	Flavonoids	Flavones-Shinoda test	+	+	-	-	+	+	+	+	+	+	-	-
		Flavanones	-	+	+	+	-	+	+	-	+	+	+	+
			+	+	+	-	-	-	+	-	+	+	+	-
		Alkaline test	+	+	+	-	+	+	+	+	+	+	+	+
4	Glycosides	Legal's test	+	-	+	-	+	+	+	+	+	+	+	+
		Bromine water test	+	+	+	+	+	+	+	+	-	-	+	+
5	Cardiac glycosides (*Keller – killani test)*		+	+	+	-	+	+	-	+	+	+	+	+
6	Terpenoids (*Salkowski's test)*		+	+	-	-	-	+	+	+	+	+	-	-
7	Phenols (*Ferric chloride test)*		+	+	+	+	+	+	+	+	+	+	+	+
8	Saponin - *Foam test*		+	+	+	+	+	-	+	+	-	+	+	+
9	Proteins	*Millon's*	+	+	+	+	+	+	+	+	+	+	+	+
		Biuret	+	+	+	+	+	+	+	+	+	+	+	+
		Xanthoproteic test	+	+	+	+	+	+	+	+	+	+	+	+
		Ninhydrin test	+	+	+	+	+	+	+	+	+	+	+	+
10	Diterpenes - copper acetate test		-	-	+	+	-	-	-	-	+	+	+	+
11	Phytosterols	Libermann Burchard's test	+	+	+	+	-	+	-	+	-	+	-	+
12	Phlobatanins		+	-	-	+	-	-	+	-	-	-	-	-
13	Tannin	Gallic tannins	+	+	+	+	+	+	+	+	+	+	-	-
14	Quinones		-	-	-	-	+	+	+	+	+	+	+	-
15	Anthroquinone (Borntragor's test)		+	+	-	-	+	+	+	+	-	-	-	+
16	Sterols		-	-	-	-	-	-	-	-	-	-	-	-
17	Triterpenoids		+	+	-	-	+	+	+	+	+	+	+	+
18	Oxalate		+	+	+	+	+	+	-	+	-	-	-	-
19	Anthocyanin		-	-	+	+	+	+	+	+	-	-	-	-
20	Vitamin C		+	+	+	+	+	+	+	+	+	+	+	+
21	Catechin		+	+	+	+	+	+	+	+	+	+	+	+
22	Resin		+	+	+	+	+	+	+	+	-	-	-	-
23	Steroids		+	+	+	+	+	+	+	-	+	+	+	-
24	Reducing sugar (Benedict's test)		+	+	+	+	+	+	+	+	-	+	+	-
25	Emodins		-	+	+	+	+	+	-	+	+	-	+	-

1 - *Acacia mearnsii* 2 - *Bracteantha bracteata* + Presence
3 - *Grevillea robusta* 4 - *Passiflora tarminiana* - Absence

Five australian species of *Acacia* were studied by in various solvents (hexane, dichloromethane, methanol, water) which they observed the presence of Alkaloids, flavonoids, tannins, anthroquinone glycosides, carbohydrates, terpenoids, saponins and steroids. They contain a variety of bioactive components such as flavonoids, alkaloids, tannins and phenolic acids. The most prominent substances in many Acacia species, however, are complex phenolic compounds (condensed tannins) and polysaccharides or gums (Maslin and Stirton, 1997).

Preliminary study conducted by Phamiwon and Shella, (2017) indicated the presence of phytochemicals like steroids, alkaloids, flavonoids, saponins, tannins, polyphenols and terpenoids in leaf extract of Passiflora edulis. *P. edulis* fruit phytochemical screening was done by few researchers and reported that Aqueous, chloroform and methanolic extracts of Passiflora edulis were found to possess tannins, flavonoids, terpenoid, steroids (Jagessar, 2017; Razia *et al*., 2014 and Nayak *et al*., 2012).

Ethyl acetate stem extraxts showed the presence of carbohydrates, alkaloids, flavonoids, glycosides, cardiac glycosides, phenols, saponin, protein, diterpenes, phlobatanin, tannin, anthroquinone, anthocyanin, vitamin C, catechin, resin, reducing sugar and absence of quinines and sterol were found in all the four plants. But few compounds were absent in one or two plants they are as follows: phytosterol absent in *A. mearnsii*; terpenoids were absent in *B. bracteata* and *P. tarminiana*; triterpenoids were absent in *B. bracteata, G. robusta* and *P. tarminiana*; Oxalate absent in *A. mearnsii, B. bracteata* and *G. robusta*; steroids absent in A. mearnsii and B. bracteata and Emodins absent in *A. mearnsii* and *P. tarminiana.*

Methanolic extract of stem samples proved to show positive sign in all the four plants for carbohydrate, alkaloids, flavonoids, glycosides, cardiac glycosides, terpenoids, phenol, saponin, phytosterol, tannin, triterpenoids, vitamin C, catechin, resin, steroids and reducing sugar while negative results in all the four plants for phlobatannins and sterols. In methanolic stem extracts few plants showed absence of certain phytoconstituents. Diterpenoid absent in *A. mearnsii* and *G. robusta*; Anthroquinone absent in *B. bracteata*; Oxalate and anthocyanin was absent in *B.bracteata, G. robusta* and *P. tarminiana* and emodins were found to be absent in *A. mearnsii* and *B. bracteata*.

Aqueous stem extracts of the four plants revealed the presence of few compounds in all the four plants as mentioned below, carbohydrate, alkaloid, flavonoid, glycosides, cardiac

Table:3 Qualitative analysis of phytoconstituents in stem extracts of *A. mearnsii, B. bracteata, G. robusta and P. tarminiana*

S.no	Test		Ethyl Acetate				Methanol				Aqueous			
			1	2	3	4	1	2	3	4	1	2	3	4
1	Carbohydrate (*Molisch's Test)*		+	+	+	+	+	+	+	+	+	+	+	+
2	Alkaloids	*Mayer's*	+	+	+	+	+	+	+	+	+	+	+	-
		Wagner's	+	+	+	+	+	+	+	+	+	+	+	+
		Hager's test	+	+	+	+	+	+	+	+	+	+	+	+
3	Flavonoids	Flavones-Shinoda test	+	+	-	-	+	-	+	-	+	+	-	-
		Flavanones	-	+	+	+	-	+	+	-	+	+	+	+
			+	+	+	-	-	-	+	+	+	+	-	+
		Alkaline test	-	+	+	+	+	+	+	-	+	+	+	+
4	Glycosides	Legal's test	-	+	+	-	+	+	+	+	+	+	+	+
		Bromine water test	+	+	+	+	+	-	+	-	+	+	-	-
5	Cardiac glycosides (*Keller – killani test)*		+	+	+	+	+	+	+	+	+	+	+	+
6	Terpenoids (*Salkowski's test)*		+	-	+	-	+	+	+	+	+	-	-	-
7	Phenols (*Ferric chloride test)*		+	+	+	+	+	+	+	+	+	+	+	+
8	Saponin - *Foam test*		+	+	+	+	+	+	+	+	+	+	+	+
9	Proteins	*Millon's*	+	+	+	+	+	+	+	+	+	+	+	+
		Biuret	+	+	+	+	+	+	+	+	+	+	+	+
		Xanthoproteic test	+	+	+	+	+	+	+	+	+	+	+	+
		Ninhydrin test	+	+	+	+	+	+	+	+	+	+	+	+
10	Diterpenes - copper acetate test		+	+	+	+	-	+	-	+	+	+	+	+
11	Phytosterols	Libermann Burchard's test	-	+	+	+	-	+	-	+	-	-	-	+
12	Phlobatanins		+	+	+	+	-	-	-	-	-	-	-	-
13	Tannin	Gallic tannins	+	+	+	+	+	+	+	+	+	+	+	+
14	Quinones		-	-	-	-	-	+	+	-	-	+	+	-
15	Anthroquinone (Borntragor's test)		+	+	+	+	+	-	+	+	-	-	-	-
16	Sterols		-	-	-	-	-	-	-	-	-	-	-	-
17	Triterpenoids		+	-	-	-	+	+	+	+	+	+	+	+
18	Oxalate		-	-	-	+	+	-	-	-	-	-	-	-
19	Anthocyanin		+	+	+	+	+	-	-	-	-	-	-	-
20	Vitamin C		+	+	+	+	+	+	+	+	+	+	+	+
21	Catechin		+	+	+	+	+	+	+	+	+	+	+	+
22	Resin		+	+	+	+	+	+	+	+	-	-	-	-
23	Steroids		-	-	+	+	+	+	+	+	+	+	+	+
24	Reducing sugar (Benedict's test)		+	+	+	+	+	+	+	+	+	-	+	+
25	Emodins		-	+	+	-	-	-	+	+	+	+	+	-

1 - *Acacia mearnsii* 2 - *Bracteantha bracteata* + Presence
3 - *Grevillea robusta* 4 - *Passiflora tarminiana* - Absence

glycoside, phenols, saponin, proteins, diterpenes, tannin, triterpinoid, vitamin C, catechin, steroids while phlobatanin, anthroquinone, sterols, oxalate, anthocyanin and resin were found to be absent. Few compounds like terpenoid absent in *B. bracteata, G. robusta* and *P. tarminiana*; phytosterol absent in *A. mearnsii, B. bracteata and G. robusta*, Quinone absent in *A. mearnsii and P. tarminiana*; reducing sugar absent in B. bracteata and emodins absent in *P. tarminiana.*

Acacia species are rich sources of bioactive compounds including biophenols, alkaloids, saponins, terpenoids, sterols, polysaccharides, non-protein amino acids, fatty acids and miscellaneous organic acids. More than one hundred flavonoids including flavans, flavanones, flavonols, flavones and their glycosides have been found in Acacia species (Subhan, 2016)

4.3 Quantitative test

4.3.1 Biochemical parameters

4.3.1.1 Carbohydrates

Maximum Carbohydrate in leaf was observed when Ethyl acetate (98.1±0.005 mg/100g of extract), methanol (41.81±3.14 mg/100g of extract) and in aqueous (32.659±2.04 mg/g of extract) was used as extraction solvent in *P. tarminiana , G. robusta* and *G. robusta* respectively. Minimum Carbohydrate in leaf when ethyl acetate (48.4±0.20 mg/100g of extract), methanol (35.52±5.38 mg/100g of extract) and aqueous (25.73±0.63 mg/100g of extract) was used as extraction solvent in *G. robusta, A. mearnsii* and *P. tarminiana* respectively.

Maximum Carbohydrate in stem was observed when Ethyl acetate (95.5±0.01mg/100g of extract), methanol (40.496±0.23mg/100g of extract) and in Aqueous (40.80±0.44 mg/100g of extract) was used as extraction solvent in *A. mearnsii, P. tarminiana* and *P. tarminiana* respectively. Minimum Carbohydrate in stem when ethyl acetate (64.5±0.05 mg/100g of extract), methanol (37.85±0.11 mg/100g of extract) and in aqueous (37.48±0.88 mg/100g of extract) was used as extraction solvent in *G. robusta, G. robusta* and *A. mearnsii* respectively.

Farid *et al*, (2014) observed carbohydrate content in three different *Acasia* species that is *A. nilotica, A. seyal* and *A. laeta.* They observed that *A. laeta* shows high content of

Table:4 Quantitative test for Biochemical parameters – Carbohydrate (mg/100g)

Plant Name	Leaf			Stem		
	Ethyl Acetate	Methanol	Aqueous	Ethyl Acetate	Methanol	Aqueous
A. mearnsii	94.6±0.03	35.52±5.38	30.25±5.41	95.5±0.01	40±1.84	37.48±0.88
B. bracteata	88.2±0.09	37.67±0.94	25.738±0.78	83.8±0.09	39.19±1.10	40.01±0.12
G. robusta	48.4±0.20	41.81±3.14	32.659±2.04	64.5±0.05	37.85±0.11	38.88±0.04
P. tarminiana	98.1±0.005	40.36±1.94	27.595±0.63	92.4±0.01	40.496±0.23	40.80±0.44
SEd	0.53930					
CD (p<0.05)	1.08439					

Values are mean ± SD of three samples in each group

Table:5 Quantitative test for Biochemical parameters – Protein (mg/100g)

Plant Name	Leaf			Stem		
	EthylAcetate	Methanol	Aqueous	Ethyl Acetate	Methanol	Aqueous
A. mearnsii	552.7±2.33	568.20±7.54	92.59±56.29	387.7±80.4	300.61±3.26	45.27 ± 3.09
B. bracteata	752.1±22.01	332.71±8.40	34.26± 11.52	995.4±0.82	213.99±4.01	13.68 ± 4.02
G. robusta	924.0±7.56	504.32±2.83	19.75±92.32	907.4±8.35	281.07±8.03	16.17 ± 1.87
P. tarminiana	858±12.14	852.16±8.99	25.56± 6.82	723.2±4.45	209.46±4.11	13.80 ± 1.16
SEd **CD (p<0.05)**	8.22731 16.54300					

Values are mean ± SD of three samples in each group

Fig 1. Comparision of Total Carbohydrates in leaf and stem Ethyl acetate, Methanol and Aqueous extracts of *A. mearnsii, B. bracteata, G. robusta* and *P. tarminiana*

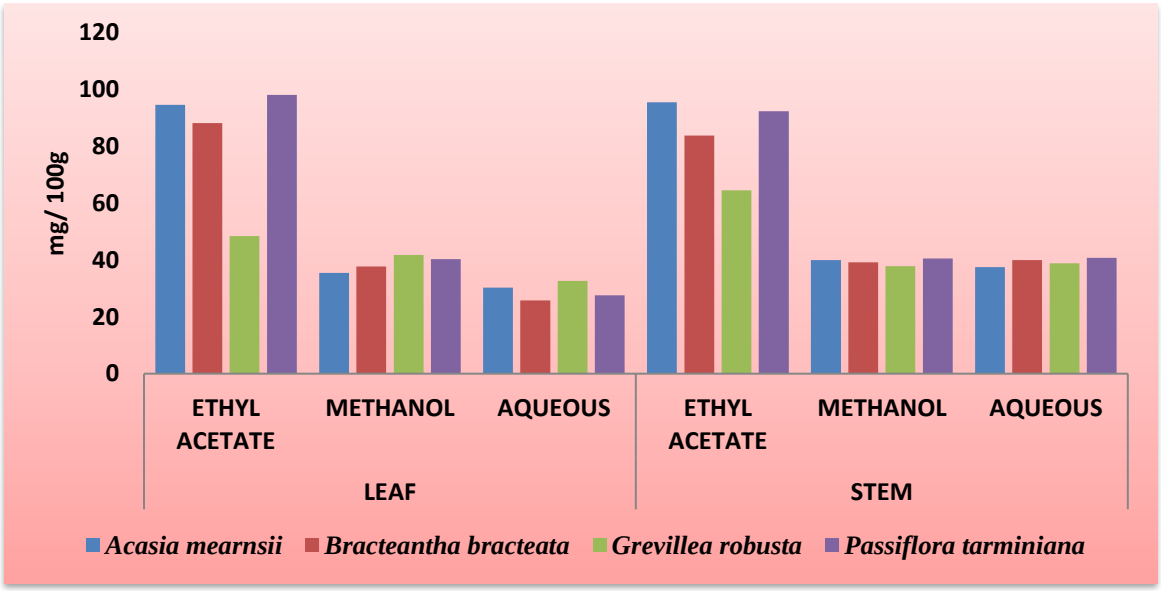
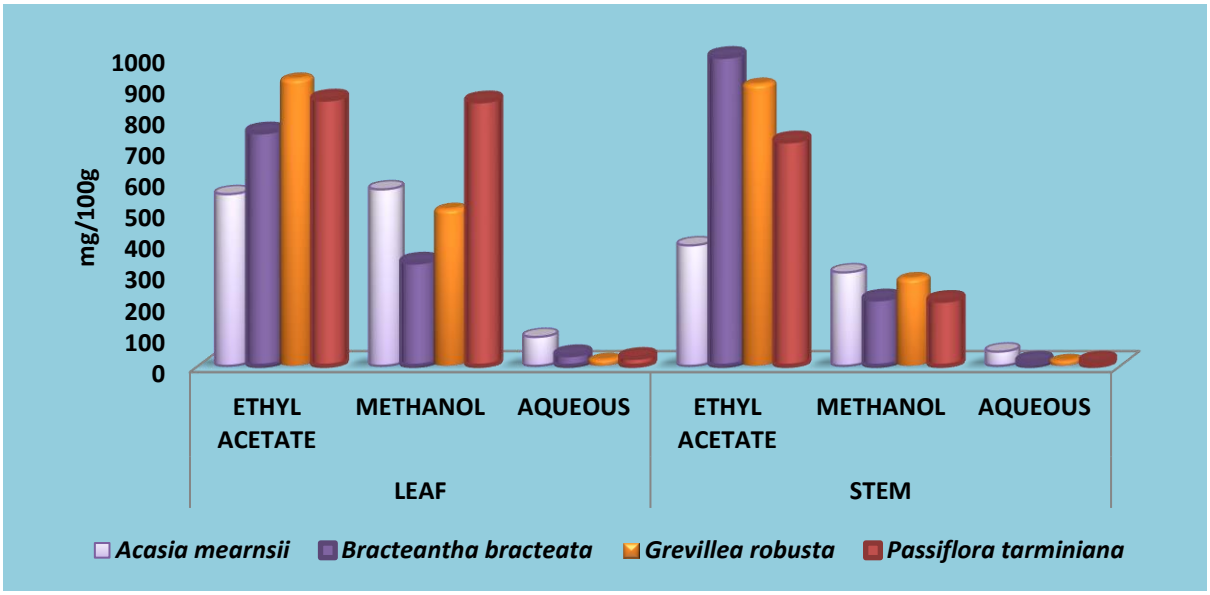


Fig 2. Comparision of Protein in leaf and stem Ethyl acetate, Methanol and Aqueous extracts of *A. mearnsii, B. bracteata, G. robusta* and *P. tarminiana*



carbohydrates in leaves (33.40±0.771mg/g), flowers (29.83±2.738mg/g) and pods (42.69±0.708mg/g).

4.3.1.2 Proteins

Maximum Protein in leaf was observed when Ethyl acetate (924.0±7.56 mg/g of extract), methanol (852.16±8.99mg/100g of extract) and in aqueous (92.59±56.29 mg/100g of extract) was used as extraction solvent in *G. robusta*, *P. tarminiana* and *A. mearnsii* respectively. Minimum protein in leaf when ethyl acetate (552.7±2.33 mg /100g of extract), methanol (332.71±8.40 mg /100g of extract) and aqueous (19.75±92.32 mg /100g of extract) was used as extraction solvent in *A. mearnsii*, *B. bracteata* and *G. robusta* respectively.

Maximum Protein in stem was observed when Ethyl acetate (995.4±0.82 mg /100g of extract), methanol (300.61±3.26 mg /100g of extract) in aqueous (45.27 ± 3.09 mg /100g of extract) was used as extraction solvent in *B. bracteata, A. mearnsii* and *A. mearnsii* respectively. Minimum protein in stem when ethyl acetate (387.7±80.4 mg /100g of extract), methanol (209.46±4.11 mg /100g of extract) and in aqueous (13.68 ± 4.02 mg /100g of extract) was used as extraction solvent in *A. mearnsii* , *P. tarminiana* and *B. bracteata* respectively.

Protein content in three different *Acasia* species (*A. nilotica*, *A. seyal* and *A. laeta*), were analysed by Farid *et al*, (2014) and observed that *A. nolotica* have highest protein content in leaves (75.42±2.409 mg/g), flowers (77.41±0.805 mg/g) and pods (61.87±3.909 mg/g) respectively.

4.3.1.3 Chlorophyll content

The chlorophyll content of the four leaf samples were obtained with the help of hand held apparatus – Plant nutrient analyser. The chlorophyll content of the plants are expressed as Soil Plant Analysis Development (SPAD) units. *A. mearnsii* was found to contain more (42.166±2.013 SPAD) while it is low (6.8±0.7 SPAD) in *P. tarminiana.*

Sharma and his co workers (2017) studied the effect of pollution on total chlorophyll content of some selected plants species along the National Highway 5. *G. robusta* which was one among the six commonly grown plants near roadside showed 2.15 mg/g of total chlorophyll content.

Table:6 Chlorophyll and Nitrogen content in leaf of *A. mearnsii, B. bracteata, G. robusta* and *P. tarminiana*

Plants	Nitrogen (mg/g)	Chlorophyll (spad)
A. mearnsii	15.466±2.557	42.166±2.013
B. bracteata	7.533±0.568	12.6±0.818
G. robusta	10.666±1.106	26.5±2.260
P. tarminiana	7.166±2.150	6.8±0.7
SEd **CD (p<0.05)**	0.917845 0.0899	0.803113 0.0652

Spad = Soil Plant Analysis Development

4.3.1.4 Nitrogen content

Nitrogen content for the four plants were analysed with the help of a plant nutrient analyser which is a hand held device used to measure Nitrogen and chlorophyll from fresh leaf samples. Among thefour samples *A. mearnsii* was found to containe more (15.466±2.557 mg/g) Nitrogen content, while less (7.166±2.150 mg/g) in *P. tarminiana*. Both nitrogen and chlorophyll content was found to be more in *A. mearnsii* and less in *P. tarminiana.* Chlorophyll and Nitrogen are dependent on each other. Assessment of one will detect content of other (Sahurkar and Chilke, 2017).

The nitrogen content of *A. senegal* found to be in the range of 0.32-0.35% and the value of 0.21% was reported for *A. seyal*, for *A. polyacantha* the nitrogen content was determined as 0.33-0.36% and that for *A. laeta* were reported as 0.31-0.32% (Naima *et al*., 2012)

4.3.2 Phytochemical parameters

4.3.2.1 Total Phenol content

Plant phenols are important bioactive constituents and they constitute the largest group of secondary metabolites with widespread presence in the plant kingdom (Quideau *et al*., 2011). The phenolic contents responsible for their antioxidant properties (Xiong *et al.*, 2016).

Fig 3. Graphical representation of Chlorophyll content and Nitrogen in fresh leaf of *A. mearnsii, B. bracteata, G. robusta* and *P. tarminiana*

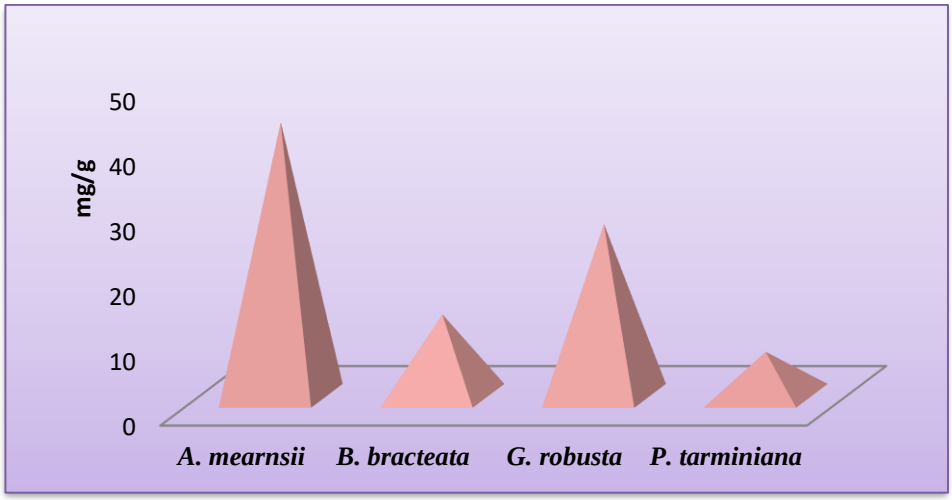
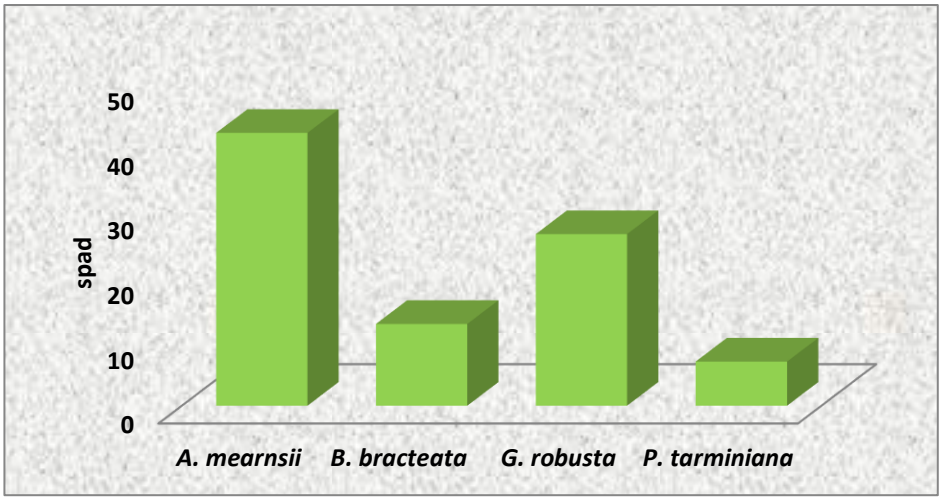


Fig 4. Graphical representation of Chlorophyll content and Nitrogen in fresh leaf of *A. mearnsii, B. bracteata, G. robusta* and *P. tarminiana*



The phytochemical screening revealed the presence of phenol. Folin-Ciocalteu method was used to measure the total phenol content and data was expressed as mg gallic acid equivalent per g dry weight (mg GAE/g)..

Maximum Phenol content in leaf was observed when Ethyl acetate (845.66±119.02 µg/ml), methanol (173.00±76.25 µg/ml) and in aqueous (115.133±10.90 µg/ml) was used as extraction solvent in *B. bracteta, P. tarminiana* and *G. robusta* respectively. Minimum phenol in leaf when ethyl acetate (188.00±42.00 µg/ml), methanol (74.33±4.04 µg/ml) and in aqueous (23.266±2.40 µg/ml) was used as extraction solvent in *A. mearnsii, G. robusta* and *B. bracteata* respectively.

Maximum Phenol content in stem was observed when Ethyl acetate (1197±195.76 µg/ml), methanol(228.6±18.42 µg/ml) and in aqueous (218.33±37.23 µg/ml)was used as extraction solvent in *G. robusta, A. mearnsii* and *P. tarminiana* respectively. Minimum phenol in stem was found in *B. bracteata* when ethyl acetate(329.33±326.47µg/ml), methanol (208.26±74.66µg/ml) and in aqueous (125.53±2.53µg/ml)was used as extraction solvent.

Olajuyigbe and Afolayan , (2011) have done the preliminary phytochemical screening in *A. mearnsii* using different solvents for extraction. Their results revealed that the acetone extract had the highest total phenolic content (47.88 mg g^{-1}). In *P.subpeltata* the total phenolics in methanol was 115.70±3.60mg/g extract equivalent to gallic acid (Saravanan and Parimelazhagan, 2013). Methanolic extract of *A. pycnantha* leaf extracts were analysed for total phenols by Suhaan, (2016) that it was around 0.1 to 60.4 mg GAE/g DW. *A.Seyal* have highest phenolic content in their flowers, leaves and pods. It was observed by Farid and his co-workers in 2014. They reported that *A. seyal* leaves have 10.24±0.104 mg gallic acid, flowers have 9.79±0.011 mg gallic acid and pods have 10.11±0.016 mg gallic acid. Phenolic content of *Passiflora edulis* leaf was found to contain 8.3±0.22 mg GAE g−1 (Prakash *et al*., 2007). The total phenol content of the aqueous leaf extract of *H. longifolium* was 0.499 mg gallic acid equivalent/g of extract power (Aiyegoro and Okoh, 2010)

4.3.2.2 Total Flavonoid content

Maximum flavanoid content in leaf was observed when Ethylacetate (7.603±1.206µg/ml), methanol (10.866±0.023µg/ml) and in aqueous (5.47±0.308 µg/ml) was used as extraction solvent in *A. mearnsii, P. tarminiana* and *A. mearnsii* respectively.

Table: 7 Quantitative test for Biochemical parameters – Total Phenol

PlantName	Leaf			Stem		
	Ethyl Acetate	Methanol	Aqueous	Ethyl Acetate	Methanol	Aqueous
A. mearnsii	188.00±42.00	93.53±13.53	79.93±10.15	666.66±185.26	228.6±18.42	213.26±44.92
B. bracteata	845.66±119.02	100.66±2.51	23.266±2.40	329.33±326.47	208.26±74.66	125.53±2.53
G. robusta	378.33±11.93	74.33±4.04	115.133±10.90	1197±195.76	213.33±15.04	153.86±17.78
P. tarminiana	275.00±75.62	173.00±76.25	38.46±5.41	517.66±128.67	224.13±45.03	218.33±37.23
SEd **CD (p<0.05)**	28.61106 57.52947					

Phenolics expressed as mg Gallic acid equivalent/ gm of dry powder

Values are mean ± SD of three samples in each group

Minimum flavanoid in leaf when ethyl acetate (6.19±0.560µg/ml), methanol (8.58±0.398 µg/ml) and in aqueous (1.4±0.095 µg/ml) was used as extraction solvent in *G. robusta, A. mearnsii* and *P. tarminiana* respectively.

Maximum flavanoid content in stem was observed when Ethylacetate (12.2±1.967µg/ml), methanol (9.093±0.230 µg/ml) and in aqueous (3.233±0.357 µg/ml) was used as extraction solvent in *G. robusta, P. tarminiana and A. mearnsii* respectively. Minimum flavanoid in stem when ethyl acetate (5.223±0.950 µg/ml), methanol (4.733±2.309 µg/ml) and in aqueous (1.78±0.476µg/ml)was used as extraction solvent in *B. bracteata, B. bracteata* and *P. tarminiana* respectively.

A. mearnsii dried leaves were extracted with methanol by Xiong *et al*, (2016) and the crude extract indicated 43.49mg gallic acid equivalent (IQE) / g total flavanoid. Suhan, (2016) worked on five different species of Acacia and found that methanolic extract of *A. pycnantha* showed high content of total flavonoids (2.4 to 153.2 mg QE/g DW). Work done by Saravanan and Parimelazhagan, 2013 revealed that the flavanoid in methanolic extract of *P.subpeltata* was 231.31mg/g extract equivalent to Rutin. *Passiflora filipes* have the maximum different flavanoid content is follows 53% of vitexin, 33% of Orientin, 44% of isoorientin and 52% of isovitexin which were estimated by Abourashed *et al*., (2002). *A. Seyal* plant also possesses high amount of flavanoids in their flowers, leaves and pods. Farid and his co-workers in 2014, estimated the amount of flavanoids in various parts of the plant and were recorded as leaves, flowers and pods contain 48.60±1.495mg quercetin, 32.62±1.451mg quercetin and 28.25±0.398 495mg quercetin respectively. According to Aiyegoro and Okoh (2010) the total flavonoid content of the *H. longifolium* was 0.705 mg gallic equivalent/g of extract powder. Flavanoid content of *P. edulis* was observed by Ferreres *et al*, (2017) and they reported that this plant have flavanoid content of 1.05±0.03 mg g−1 in their leaves.

4.3.2.3 Tannin

Tannins, including hydrolysable and condensed tannins (proanthocyanidins), are the next leading compounds in Acacia species after flavonoids. They have also been identified in and isolated from different parts of Acacia species. Proanthocyanidins are the most conspicuous type of tannins in Acacia. Tannins are important in many foods and animal diets.

Table:8 Quantitative test for Biochemical parameters – Total Flavanoid

Plant Name	Leaf			Stem		
	Ethyl Acetate	Methanol	Aqueous	Ethyl Acetate	Methanol	Aqueous
A. mearnsii	7.603±1.206	8.58±0.398	5.47±0.308	8.496 ±1.787	5.673±0.219	3.233±0.357
B. bracteata	6.8±1.158	9.166±0.075	3.766±0.820	5.223±0.950	4.733±2.309	3.053±0.468
G. robusta	6.19±0.560	10.77±0.291	4.096±0.385	12.2 ±1.967	5.113±0.132	2±0.160
P. tarminiana	7.66±1.251	10.866 ±0.023	1.4±0.095	6.21±1.326	9.093±0.230	1.78±0.476
SEd **CD (p<0.05)**	0.27233 0.54759*					

Flavonoids expressed as mg Quercetin equivalent/ gm of dry powder

Values are mean ± SD of three samples in each group

Fig 5. Comparision of Total Phenol in leaf and stem Ethyl acetate, Methanol and Aqueous extracts of *A. mearnsii, B. bracteata, G. robusta* and *P. tarminiana*

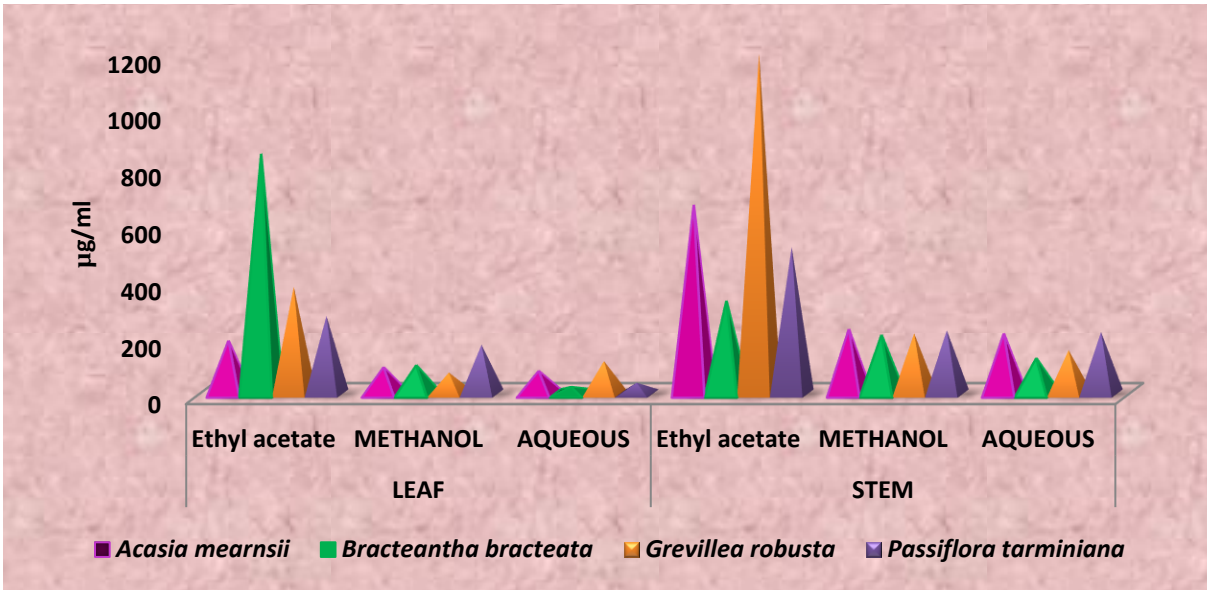
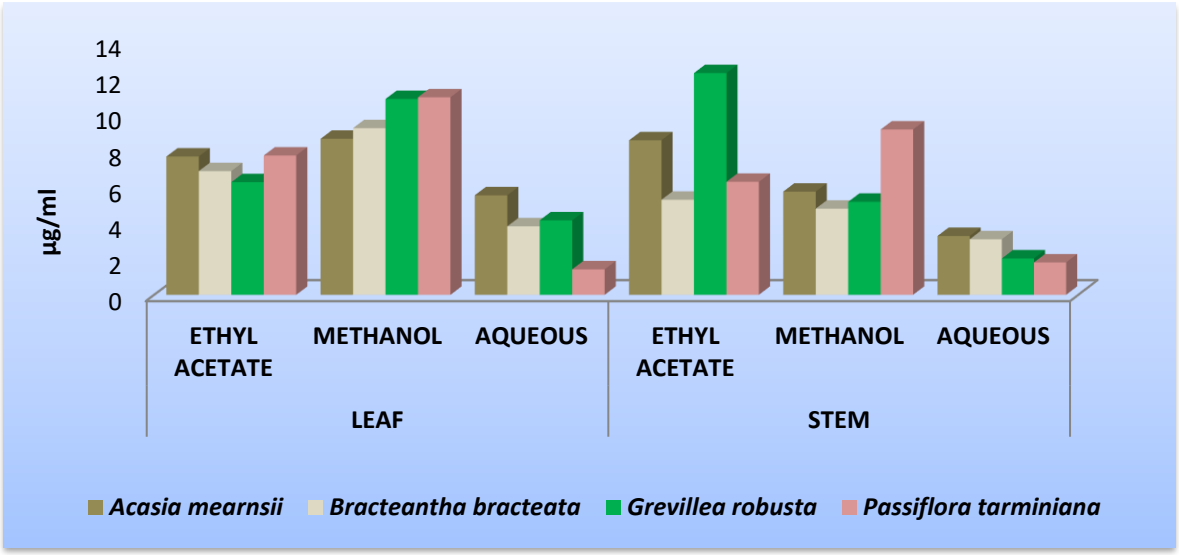


Fig 9. Comparision of Total Flavonoid in leaf and stem Ethyl acetate, Methanol and Aqueous extracts of *A. mearnsii, B. bracteata, G. robusta* and *P. tarminiana*



Maximum tannins in leaf was observed when Ethyl acetate (532±169.00µg/ml), methanol (313±0.34 µg/ml) and in aqueous (210.66±63.25µg/ml) was used as extraction solvent in *A. mearnsii, B. bracteata* and *G. robusta* respectively. Minimum tannins in leaf when ethyl acetate (244.26±22.27 µg/ml), methanol (169±143.04 µg/ml) and aqueous (100.33±5.03 µg/ml) was used as extraction solvent in *G. robusta, A. mearnsii* and *P. tarminiana* respectively.

Maximum tannins in stem was observed when Ethyl acetate (1024.33±10.01 µg/ml), methanol (442±81.54 µg/ml) and in Aqueous (162.86±14.10 µg/ml) was used as extraction solvent in *G. robusta, P. tarminiana* and *G. robusta* respectively. Minimum tannins in stem when ethyl acetate (296.8±7.86 µg/ml), methanol (174.66±110.38 µg/ml) and in aqueous (73.33±15.30 µg/ml) was used as extraction solvent in *A. mearnsii, A. mearnsii* and *G. robusta* respectively.

Presence of 39.8% tannin in *A. mearnsii* was revealed by Ahmed *et al*., (2005). According to them only condensed tannin was present in combined method (Folin – Dehis and Hagerman – Buffer method). According to Saravanan and Parimelazhagan (2013) tannin content was observed to be 60.23 mg/g equilant to gallic acid in methanolic extract. Ahmed *et al*, (2005) worked on different parts of *Acacia* species, bark, pods or leaves, had the same type of tannin but in different proportions. Usually the tannin content was higher in the deseeded pods than in the bark. The tannin content determined by the official hide-powder method which indicates the highest (39.8%) tannin content in *A. mearnsii* pods.

4.4 Antioxidant assays

4.4.1 Enzymatic antioxidants

4.4.1.1 Polyphenol oxidase

The level of peroxidise activity was found to be more in *G. robusta* leaf (60.33±24.020 U/g), while less in *A. mearnsii* (15.00±4.00 U/g).

4.4.1.2 Peroxidase

The present investigation showed the peroxidise activity to be high (76.33±4.667) in G. robusta and less (43.00±6.937) in *A. mearnsii.*

Table: 9 Quantitative test for Biochemical parameters – Total Tannin (µg /ml)

Plant Name	Leaf			Stem		
	Ethyl Acetate	Methanol	Aqueous	Ethyl Acetate	Methanol	Aqueous
A. mearnsii	532±169.00	305±10.24	139±59.80	296.8±7.86	174.66±110.38	109.33±12.05
B. bracteata	347.66±73.20	313±0.34	114.66±10.40	597.33±42.0	187.4±20.31	73.33±15.30
G. robusta	312.66±48.41	290.5±13.27	210.66±63.25	1024.33±10.01	382±50.23	162.86±14.10
P. tarminiana	244.26±22.27	169±143.04	100.33±5.03	331.33±75.87	442±81.54	149.66±28.54
SEd	18.26367					
CD (p<0.05)	36.72353					

Values are mean ± SD of three samples in each group

Fig 6. Comparision of Total Tannin in leaf and stem Ethyl acetate, Methanol and Aqueous extracts of *A. mearnsii*, *B. bracteata*, *G. robusta* and *P. tarminiana*

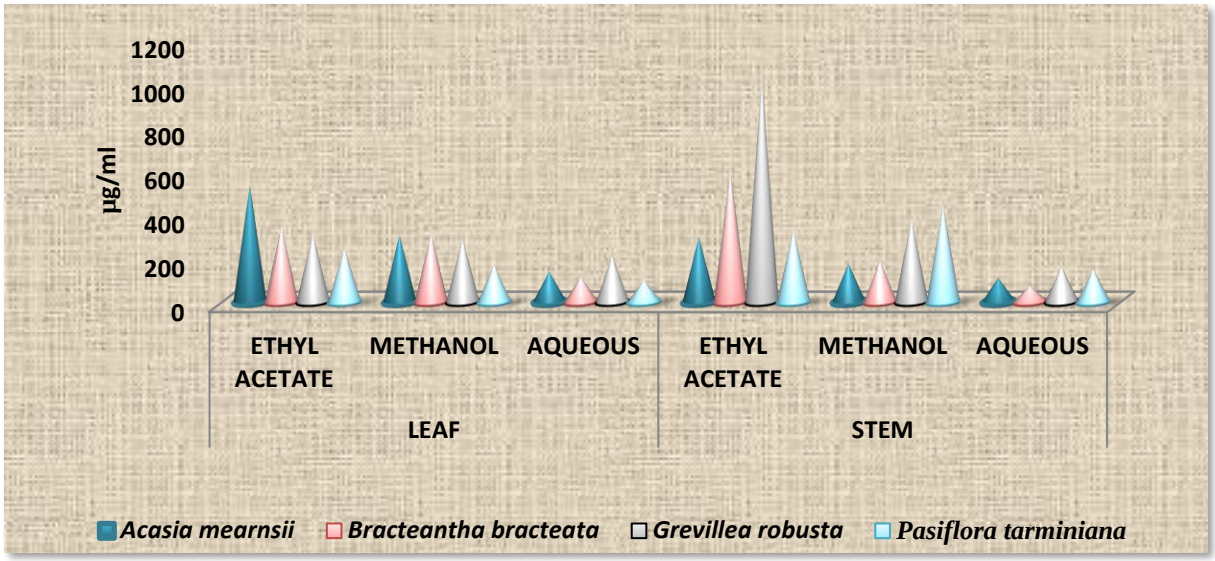


Table: 10 Levels of various antioxidant enzymes in leaf of *A. mearnsii, B. bracteata, G. robusta* and *P. tarminiana*

Plants	Polyphenol oxidase ($U^{@}$/g)	Peroxidase ($U^{\$}$/g)
A. mearnsii	15.00±4.00	43.00±6.937
B. bracteata	41.73±25.99	55.33±3.290
G. robusta	60.33±24.020	76.33±4.667
P. tarminiana	16.33±6.506	71.00±3.841
SEd **CD (p<0.05)**	11.47783 0.024*	1.605219 0.001*

Values are mean ± SD of three samples in each group

$^{@}$/ unit – Activity of catechol oxidase which transforms 1 µ mol of dihydro-phenol to quinine / minute

$^{\$}$/ unit – change in absorbance / minute at 430 nm

Table: 11 Comparision Non enzymatic antioxidants – Ascorbic acid (µg / ml)

Plant Name	Leaf			Stem		
	Ethyl Acetate	Methanol	Aqueous	EthylAcetate	Methanol	Aqueous
A. mearnsii	301±194.88	162.33±29.73	111±7.54	816.6±9.50	279.00±28.93	134.33±12.89
B. bracteata	246.66±66.10	124±8.71	145.66±33.24	216.6±20.10	650.00±75.02	590.66±30.89
G. robusta	132.33±10.01	170.33±24.21	190±32.41	226.6±5.68	756.33±18.33	389.66±31.72
P. tarminiana	225.33±119.03	113±31.22	115±11.26	361.00±5.56	634.00±84.12	292.00±4.58
SEd **CD (p<0.05)**	16.46338 33.10361					

Values are mean ± SD of three samples in each group

4.4.2 Non-enzymatic antioxidants

4.4.2.1 Ascorbic acid

Maximum Ascorbic acid in leaf was observed when Ethyl acetate (301±194.88µg/ml), methanol (170.33±24.21µg/ml) and in aqueous (190±32.41µg/ml) was used as extraction solvent in *A. mearnsii*, *G. robusta* and *G. robusta* respectively. Minimum Ascorbic acid in leaf when ethyl acetate (132.33±10.01 µg/ml), methanol (113±31.22µg/ml) and aqueous (111±7.54 µg/ml) was used as extraction solvent in *G. robusta*, *P. tarminiana* and *A. mearnsii* respectively.

Maximum Ascorbic acid in stem was observed when Ethyl acetate (816.6±9.50µg/ml), methanol (756.33±18.33µg/ml) and in Aqueous (590.66±30.89 µg/ml) was used as extraction solvent in *A. mearnsii*, *G. robusta* and *B. bracteata* respectively. Minimum Ascorbic acid in stem when ethyl acetate (226.6±5.68 µg/ml), methanol (279.00±28.93µg/ml) and in aqueous (134.33±12.89µg/ml) was used as extraction solvent in *G. robusta*, *A. mearnsii* and *A. mearnsii* respectively.

The ascorbic acid content of this fruit was reported, and the fruit was considered a rich source of vitamin C (40 mg per 100 g of edible fruit) (Valente *et al*., 2011). Wei and his co-workers have done their work on *G. robusta* they estimated that proanthocyanidin extract of *G. robusta* contains ascorbic acid (117.10 ± 1.54 µg/mL) level (Wei *et al*., 2012). Fruit is considered a rich source of Vitamin C (40mg/100g of edible fruit), which confers it good antioxidant capacity (Simirgiostis *et al*., 2013)

Fig 7. Comparision of enzymatic antioxidants in leaf of *A. mearnsii*, *B. bracteata*, *G. robusta* and *P. tarminiana*

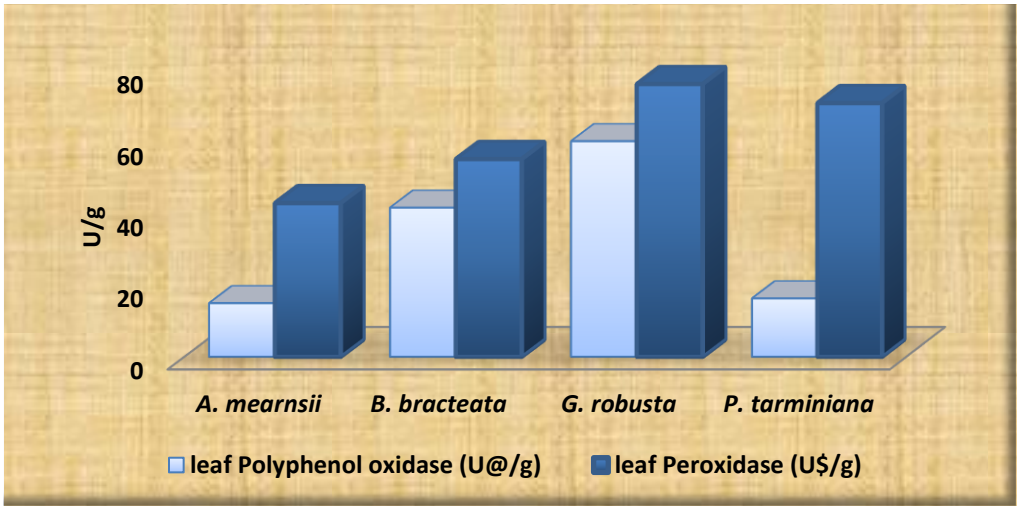
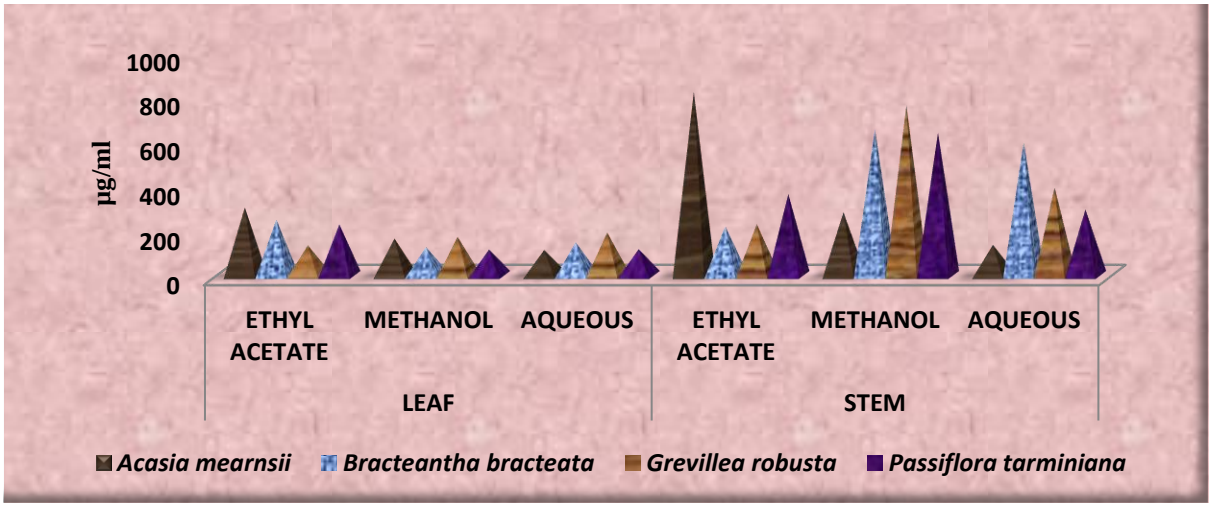


Fig 8. Comparision of Ascorbic acid in leaf and stem Ethyl acetate, Methanol and Aqueous extracts of *A. mearnsii*, *B. bracteata*, *G. robusta* and *P. tarminiana*



Summary &Conclusion

Summary and conclusion

The present study deals with the qualitative, quantitative analysis of phytochemicals in stem and leaf extracts and antioxidant activity present in leaf of *Acacia mearnsii, Bracteantha bracteata, Greviellia robusta* and *Passiflora mollisima.*

The phytochemical screening revealed the presence of carbohydrates, alkaloids, flavonoids, glycosides, phenols, proteins, vitamin C and catechin in all the three extracts of leaf samples. While in stem extracts of all the four plants carbohydrates, alkaloids, flavonoids, glycosides, cardiac glycosides, phenols, saponin, proteins, tannin, vitamin C and reducing sugar were present.

The present investigation revealed that presence of Carbohydrate in leaf to be more in Ethyl acetate extract of *P. mollisima* and less in aqueous extract of *B. bracteata*. Among the three extracts used Ethyl acetate showed more carbohydrate in all the four plants than methanol and aqueous extract. Quantitative analysis in stem showed maximum in Ethyl acetate Extract of *A. mearnsii* while minimum in aqueous extract of *G.robusta*. In overall study in leaf the order of extracts which had more and less values were Ethyl acetate > Methanol > Aqueous; While it is in the same order except aqueous extract of *P. mollisima.*

The overall results showed the estimation of protein using all the three extracts resulted in Ethyl acetate > Methanol > Aqueous in both leaf and stem. The highest value was found in *G. robusta* Ethyl acetate leaf extract While the lowest concentration was found in Aqueous extract of *G. robusta.* In stem the maximum concentration of protein was found in Ethyl acetate extract of *B. bracteata* while *B. bracteata* showed the minimum amount of protein in aqueous stem extract.

Phenols was estimated in leaf and was found to be high in Ethyl acetate extract

of *B. bracteata* and low in aqueous extract of *B. bracteata*.

The present investigation revealed in leaf the presence of flavanoids in maximum amount in methanolic extract of *P. mollisima* and minimum in aqueous extract of *P. mollisima* In stem the amount of flavanoids were observed to be more in methanolic extract of *P. mollisima* and low in aqueous extract of *P. mollisima*.

The tannin contents of three extract in stem and leaves of all the four plants were expressed as gallic acid equivalents. Among the three extracts in leaf Ethyl acetate extract of
A. mearnsii contained the highest amount of tannin while the lowest tannin content in leaf was observed in aqueous extract of *P. mollisima*. Among the four plants tannin content was observed to be more in Ethyl acetate and less in aqueous in both leaf and stem. In the the highest tannin content was observed in Ethyl acetate extract of *G. robusta*, while the lowest was found in aqueous extract of *B. bracteata*.

The present investigation revealed the presence of ascorbic acid in leaf and stem. The highest was observed in Ethyl acetate extract of *A. mearnsii* with Ethyl acetate showed maximum and minimum was observed in aqueous extract of *A. mearnsii* respectively.

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