Antioxidant and Antimicrobial Activities of *Hygrophila schulli* (Buch.-Ham.)

THULASI LAKSHMI,K.

(14PZO005)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education

for Women, Coimbatore – 641 043

In Partial Fulfilment of the Requirements for the Degree of

Master of Science in Zoology

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I. INTRODUCTION

During the past decades, the contribution of herbal medicines and their preparations has attracted much interest in the pharmaceutical industry. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases (Verpoorte, 1998). Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts (Sandhya *et al.,* 2006).

Medicinal plants are used medically in different countries and are a source of many potent and powerful drugs. Scientific investigations of medicinal plants have been initiated in many countries because of their contributions to health care (Mahesh and Sathish, 2008).

Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient times and an impressive number of modern drugs have been developed from them. The primary benefits of using plant-derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment.

The first written records on the medicinal uses of plants appeared about 2600 BC from the Sumerians and Akkaidians (Samuelsson, 1999). The ancient texts like Rigveda (4500-1600 BC) and Atharvaveda mention the use of several plants as medicine such as Charakasamhita and Susruthasamhita refer to the use of more than 70 herbs (Jain, 1968).

According to the World Health Organisation "a medicinal plant is any plant which in one or more of its organ contains substances that can be used for therapeutic processes or which are precursors for the synthesis of useful drugs". The definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation.

Furthermore, WHO defines medicinal plant as herbal preparations produced by subjecting plants materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products.

Indian medicinal plants have contributed a great deal to the academic curiosity as it is apparent from the number of publications, but could not provide breakthrough molecules such as paclitaxel and artemisinin for discovery. The credits for the leads like reserpine and forskolin earlier obtained from the plants of traditional Indian system of medicine had been taken by the western pharmaceutical companies. Still indigenous systems of medicines have a great scope for the discovery of leads for several disease classes by the virtue of the chemical and biological diversity (Bhutani and Gohil, 2010).

In India, almost 95% of the prescriptions were plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha (Sathyavati *et al*.,1987). The materia medica of these systems contains a rich heritage of indigenous herbal practices that have helped to sustain the health of most rural people of India.

Although India has rich culture of medicinal herbs and spices, and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Sidha traditional medicines, only very few have been studied chemically and pharmacologically for their potential medicinal value (Gupta *et al*., 2005). Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources (Fabricant and Farnsworth, 2001).

Hygrophila schulli (Buch.-Ham) is a thorny sub-shrub of the family *Acanthaceae* that grows widely throughout India, Sri Lanka, Myanmar, Indo-China, Tropical Africa and Malaya. The synonyms of *H. schulli* include *H. auriculata* (K. Schum) Heine and *Astercantha longifolia* (L.) Nees. The erect armed sub-shrub with purplish stem generally has eight leaves and six spines at each node. Leaves are whorled, linear-lanceolate, with undulating margins.

Flowers form in axillary sessile whorls, with leafy bracts and bracteoles, and pink corolla. Capsules are 1 cm long and seeds are orbicular.

In ayurvedic literature, it is described as Ikshura, Ikshugandha, and Kokilaksha (having eyes like the Kokila) or Indian Cuckoo. It is classified in the Ayurvedic system of medicine as Seethaveryam, Mathuravipaka and is used for the treatment of a number of conditions, including premeham (diabetes) and athisaram (dysentery) (Nadkarni, 1978; Chopra, 1986).

Traditionally, the leaves are used as/in diuretic, jaundice, antibacterial, dropsy, rheumatism, anasaraca, diseases of urinogenital tract, leucor, sweet, sour, bitter, tonic, oleaginous, aphrodisiac, hypnotic, diarrhoea, dysentery, urinary calculi, urinary discharge, anti inflammatory, joint pain, biliousness, eye disease, ascites, abdominal troubles, anaemia, anuria, gleets, cough, demulcent, stomachic, lumbago, arthritis, gastric disorder and leucorrhoea (Patra *et al*., 2009).

Phytochemicals are non-nutritive plant chemicals that contain certain protective, disease–preventing compounds. More than 900 different phytochemicals have been identified as components of food, and many more phytochemicals continue to be discovered today. Researchers have long known that there are phytochemicals present for protection in plants, but it has only been recently that they are being recommended for protection against human diseases.

The advantage of using phytochemical compounds for cancer treatment is their relatively non-toxic nature and availability in an ingestive form. Many of the phytochemicals present in human diet have been identified as potential chemo preventive agents. Hence in the present study, an attempt was made to elucidate the role of major phytochemicals present in the methanol extract of *H.schulli*.

Since the phyto-constituents and volatiles of medicinal herbs have created renewed demand in their use by the public, explorations of health benefits and antioxidant potential of these metabolites in the prevention of problems raised due to oxidative stress is needed.

Oxidative stress constitutes an alteration that is encountered when there is an imbalance between the production of ROS and the ability of the biological system to readily detoxify these reactive intermediates or easily repair the resulting

damage. Halliwell (2011) reported that the accumulation of net damage due to oxidative stress over a period of time is considered responsible for many diseases including cancers.

Antioxidants are the class of compounds having their role in free radical scavenging. These free radicals are formed as a result of various catabolic and anabolic reactions occurring in our body. They are very reactive species and have deleterious impact on the normal human cells. They cause damage to normal cells leading to abnormal functioning of the cells.

Antioxidants can act by diverse mechanisms in the oxidative sequence. The human body complex antioxidant defence system consists of the dietary intake of antioxidants, as well as the endogenous production of anti-oxidative compounds, such as glutathione, etc. (Clarkson and Thompson, 2000). Antioxidant responses of our body can accommodate increased oxidative damage in diseased states to a level, but beyond those additional antioxidants are required to combat the increased stress.

Different types of extracts are prepared from different parts of the medicinal plants which are the source of these antioxidants. The antioxidants are generally dissolved in organic or aqueous compounds used while making extracts from different parts of different medicinal plants thus becoming the good source of antioxidants which can be tested on different cancerous cell lines and later identified as proper chemical compounds.

Several studies have shown that plant derived antioxidant scavenge free radicals and modulate oxidative stress. The anticancer agents, vinblastine and vincristine from the Madagascar Periwinkle, *Catharanthus roseus* G. Don. (Apocynaceae), introduced a new era of the use of plant material as a medication for treatment. They were the first agents to advance into clinical use for the treatment of cancer. Vinblastine and vincristine are used in combination with other cancer drugs, for the treatment of various kinds of cancers, including leukemias, lymphomas, advanced testicular cancer, breast and lung cancers (Ahmed *et al*., 2013).

DPPH, a stable free radical, possesses the property of delocalization and provides a deep violet color and undimerization capacity when react with plant extracts. Antioxidants that possess electron-donating capacity reduce this radical, forming a yellow colour. Various free radicals like superoxide, nitric oxide, hydroxyl radicals are toxic ROS that have capability to disrupt various signalling pathways and can lead to cell death. Analysis between bioactive constituents and scavenging assays revealed good positive correlations that suggest the possible dependency of antioxidant activity on the presence of bioactive compounds (Apak *et al*., 2007).

The reducing capacity of the plant extracts may serve as an indicator of potential antioxidant activities through the action of breaking the free radical chain by donating hydrogen atom (Jamuna *et al*., 2011). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Chanda and Dave, 2009). In the present investigation, an attempt was made to evaluate the antioxidant potential of *H.schulli* by measuring the scavenging (DPPH) and reducing power abilities.

The demand for plant based medicines and other herbal healthcare products, including pharmaceuticals, food supplements (functional foods), cosmetics (cosmeceuticals), etc. is increasing steadily in both developing and developed countries due to the growing recognition that the natural products are relatively non-toxic, have less side effects and easily available at affordable prices (Nair and Shaji, 2008).

Botanicals or phyto-pharmaceuticals are very suitable for prophylactic use in order to prevent diseases and also to maintain our normal wellbeing (Hussain *et al*., 2010). The extensive use of synthetic compounds led to a decline in the use of plants in modern medicine; however, synthetic drugs often cause considerable side effects, and as a result, people are more favouring the use of natural compounds obtained from plants (Dalal and Sudhir, 2010). In the present investigation, an attempt was made to elucidate the chemical nature of the methanol extract of *H.schulli* by means of spectroscopic and XRD analysis.

Medicinal plants are a rich source of secondary metabolites and they are exploited from time immemorial as powerful drugs in traditional/alternative healthcare systems. Many pharmaceutical companies are showing great interest in plant derived drugs mainly due to the current widespread belief that 'Green Medicine' is effective, safer and more reliable than synthetic drugs (Sujatha, 2005).

Essential oils are complex mixtures of volatile organic compounds produced as an end product of secondary metabolism. These oils with standardized content of components have certain chemicals which determine the therapeutic quality. Each component of the essential oils contributes to the beneficial or adverse effects of these oils as the component of each essential oil has different properties and bio availabilities. In the present investigation, the bioactive components from the methanol extract of *H.schulli* were identified by Gas chromatography mass spectrometry analysis.

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day (Yadav and Khan, 2012). Several synthetic antibiotics and drugs are employed in the treatment of the microbial infections and communicable diseases; but, the microbial pathogens develop resistance to the synthetic antibiotics. The increasing incidence of resistance to antibiotics and their side effects on the functioning of different parts of the body organ systems necessitate to finding out substitutes for the antibiotics (Sasikumar *et al*., 2007).

In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are often found with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs (Babu and Subha sree, 2009).

Among many proposed strategies, a good understanding of systematic screening of traditional system of medicine offers the potential of developing

potent broad spectrum antibiotics. The resistance of pathogenic microorganisms to currently known antibiotics is constantly increasing due to a broad use of antimicrobials in medicine, animal husbandry and agriculture. These documents opens up an expanding spectrum of applications and better understanding of diagnostic and therapeutic purposes to get effective antimicrobial agents..

It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants and out of the 4,00,000 plant species on earth, only a small number has been systematically investigated for their antimicrobial activities (Anjana *et al*., 2009).

Most of the synthetic drugs cause side effects and also most of the microbes developed resistant against the synthetic drugs (Chanda and Rakholiya, 2011). To alleviate this problem, antimicrobial compounds from potential plants should be explored. These drugs from plants are less toxic; side effects are scanty and also cost effective. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Harishchandra *et al.,* 2012). Hence, in this present study, same efforts are continued in the progression of searching novel therapeutics.

The present investigation was carried out to screen the antibacterial and antifungal properties of methanol extract of *H. schulli* collected from Kuttanad wetlands, Kerala state, India against pathogenic bacteria and fungi,

In view of the above, the present investigation was undertaken with the following objectives.

The objectives are

- To analyse the phytochemical constituents of methanolic extract of *Hygrophila schulli*.
- To undertake chemical characterisation of the active components present in the methanolic extract of *Hygrophila schulli.*
- To identify the secondary active constituents from the methanolic extract of *Hygrophila schulli* .
- To assess the *in vitro* antioxidant potential of methanolic extract of *Hygrophila schulli*.
- To evaluate the antimicrobial activity of methanol extract of *Hygrophila schulli*.

II. REVIEW OF LITERATURE

The review of literature pertaining to the topic **"Antioxidant and antimicrobial activities of *Hygrophila schulli* (Buch.-Ham.)"** is presented in the following headings:

2.1. Natural plant product research

In the 21st century, finding and developing new drugs from natural plants have attracted more and more attention (Wang *et al*., 2012). Various plant medicines and health products have been accepted by people from all over the world, looking forward to improving the quality of life, disease prevention and treatment of chronic diseases and geriatric diseases as well as western medicine with helpless mysterious illness.

Natural products derived from fruits, vegetables, herbs and marine products have served us well in combating cancer. The compounds are well characterized as possessing a wide variety of anti- tumour properties. Active ingredients such as alkaloids, flavonoids, terpenoids, polysaccharide and saponin obtained from natural products have potent biological properties such as anti- tumour, analgesia, anti- inflammatory, immune modulation and anti- viral activities.

Historically, natural products in the field of anti- cancer research has made significant achievements; over 60% of the clinical use of anti- cancer drugs originate from natural products (Seelinger *et al.,* 2012), including plants, marine organisms and microbes. Plants have been the main resources in traditional medicine and natural products are considered as important sources of anti- tumour drugs.

Medicinal plants play a vital role for the development of new drugs (Sharma and Agrawal, 2014). The World Health Organization has defined traditional medicine as comprising therapeutic practices that have been in existence for hundreds of years (Kamboj, 2002). The traditional preparations comprise medicinal plants, minerals and organic matter. Herbal drugs constitute only those

traditional medicines which primarily use medicinal plant preparations for therapy (Hota and Pathi, 2003).

In countries with poor economics like India, it is useful to employ a number of indigenous plant medicines due to the relatively high cost of allopathic medicines (Okeke *et al*., 1999). Due to extensive folk medicine systems like ayurveda and siddha which have existed since prehistoric times, India is blessed with a wealth of ancestral knowledge about such plants. However, to ensure safety and efficacy, the crude drugs derived from plants must be subjected to extensive phytochemical analysis to develop sustainable, safe and marketable drugs from them (Odebiyi *et al*., 1978).

Over the past 25 years the U.S. National Cancer Institute has made an attempt at testing and studying the anti-tumour activity of some 114,000 plants representing 40,000 species. To date about 4-5% of these extracts have shown reproducible activity by isolating specific substances such as alkaloids, terpenoids and phenols.

2.2. *Hygrophila schulli* - An overview

Hygrophila schulli Buch.-Ham. (Fam.: Acanthaceae) is an annual erect, unbranched, semi-aquatic herb. *H. schulli* is commonly found associated with and wetlands, which forms large stands by easily colonizing in waterlogged areas. Its common habitats are moist places, on banks of ponds, ditches and paddy fields.

The plant is widely distributed throughout Indo-China, Myanmar, India, Nepal, Sri Lanka, Pakistan, Tropical Africa and Bangladesh. Due to its wide availability and enormous medicinal values, ethnic people of Bangladesh have been using this plant from the time unknown. The ethnic people of Patuakhali district of Bangladesh use fresh aerial parts of the plant and ash in dropsy. The Garo tribe living in Mymensingh district of Bangladesh uses the plant in diarrhea, dysentery, and cough.

In India, it is being used as vegetable in some states like Odisha, Chhattisgarh and West Bengal. The pre-flowering or flowering succulent aerial

parts are boiled and consumed by the rural people of these states to increase the haemoglobin level. This herbal remedy does not have any side effects with proven effectiveness.

The roots and leaves are diuretic and used in the treatment of dropsy, jaundice and urinogenital diseases. Seed juice is useful to mother during child birth. On the other hand the leaf paste is applied externally to lumbago and rheumatism (Ahmed *et al*. 2007 - 2009).

Hygrophila schulli (Buch. – Ham.) is described in ayurvedic literature as Ikshura, Ikshugandha, and Kokilasha, as like having eyes as Indian Cuckoo. Also classified in ayurvedic system as seethaveeryam, mathuravipaka and used for the treatment of premeham (Diabetes), athisaram (Dysentry) etc. (Nadkarni, 1978; Chopra *et al*., 1986).

The whole plant, roots, seeds, and ashes of the plant are extensively used in traditional system of medicine for various ailments like rheumatism, inflammation, jaundice, hepatic obstruction, pain, urinary infections, oedema and gout.The plant has been reported to be used in dropsy, diarrhoea, dysentery, cough, jaundice, and urinogenital diseases. Seed juice of the plant was found to be useful during child birth; leaf paste was reported to be applied externally to lumbago and rheumatism (Asiatic Society, 2010).

2.3. Phyto chemicals and its significance

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. These phytochemicals were used to cure the disease in herbal and homeopathic medicines. These are non-nutritive substances, have protective or disease preventive property. With advances in phytochemical techniques, several active principles of many medicinal plants have been isolated and introduced as a valuable drug in modern systems of medicine (Hemalatha *et al.,* 2013).

Phytochemicals are associated with the prevention and treatment of at least four of the leading causes of death - cancer, diabetes, cardiovascular disease and

hypertension. They are involved in many processes including once that help prevent cell damage, prevent cancer cell replication, and decrease cholesterol levels. With health- care costs being a major issue today, it would be cost effective to continue the research needed to help promote the awareness and consumption of phytochemicals as a prevention strategy for the public.

Alkaloids are a diverse group of low molecular weight, nitrogen-containing compounds mostly derived from amino acids. Alkaloids are thought to play a defensive role in the plant against herbivores and pathogens. Plant-derived alkaloids currently in clinical use include analgesics, anti- neoplastic agent, gout suppressant, muscle relaxants, antiviral, cytotoxic, anticholinergic, anti-inflammatory and DNA-binding activities and some of them have also been used in the treatment of Alzheimer's disease, myasthenia gravis and myopathy (Crozier *et al*., 2006).

Flavonoids are a group of poly phenolic compounds, which are widely distributed throughout the plant kingdom. Many have low toxicity in mammals. Flavonoids exhibit several biological effects such as anti- inflammatory, anti-hepatotoxic and anti-ulcer actions. They also inhibit enzymes such as aldose reductase and xanthine oxidase.

They are potent antioxidants and have free radical scavenging abilities. They may prevent production of oxidants, inhibit oxidants from attacking cellular targets, block propagation of oxidative reactions and reinforce cellular antioxidant capacity. Flavonoids also possess anti- inflammatory and anti-platelet aggregation effects through inhibiting relevant enzymes and signalling pathways, resulting ultimately in lower oxidant production (Hazra *et al.,* 2008).

Flavonoids of medicinal plants are proposed as an antitumor agent in various researches. They have been shown to inhibit the growth of various cancer cell lines *in vitro*, and reduce tumour development in experimental animals. A large clinical study, in which 9959 men and women were followed for 24 years, showed an inverse relation between the intake of flavonoids (for example quercetin) and lung cancer. One possible explanation for these conflicting data is

that flavonoids are toxic to cancer cells or to immortalized cells, but are not toxic or are less toxic to normal cells (Nijveldt *et al*., 2001).

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al.*, 2007). They possess biological properties such as anti-apoptosis, anti-aging, ant carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al*., 2007).

Steroids have been reported to have antibacterial properties (Raquel, 2007) and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001).

Variety of saponins with complex structure widely exists in plants. The most widely studied is the ginsenoside. Ginsenoside is a kind of triterpenoidsaponins, and it is the main active ingredients in ginseng. A large number of studies have shown that ginsenoside has higher anti- tumour activity, non- toxic side effects on normal cells and has a synergistic effect with other chemotherapy drugs such as cisplatin. Ginsenoside regulates the proliferation of tumour cells, inducing differentiation and apoptosis of cells to exert anti- tumour effects.

2.4. Major nutrient and chemical constituents of *H. schulli*

Medicinal plants therapeutic value can be evaluated on the basis of presence of chemical substances producing definite physiological action on human body involved in the defense mechanism of plants called phytochemicals. These photochemical are of different categories like Tannins, Cardiac glycosides, Phenolics, Flavanoids, Alkaloids, Anthocyanins, Essential oils, Terpenoids etc which got extracted to organic and aqueous phases according to their polarity while making extracts of different medicinal plants.

The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. It has been suggested that there is an inverse

relationship between dietary intake of antioxidant rich foods and incidence of human diseases (Yildrim *et al*., 2001).

Plant based antioxidants are preferred to the synthetic ones because of their multiple mechanisms of actions and non-toxic nature. These facts have inspired widespread screening of plants for possible medicinal and antioxidant properties; the isolation and characterization of diverse phytochemicals and the utilization to antioxidants of natural origin to prevent the diseases (Akinmoladun *et al*., 2007)

H.schulli contains various groups of phyto-constituents viz. phytosterols, fatty acids, minerals, polyphenols, pro anthocyanins, mucilage, alkaloids, enzymes, amino acids, carbohydrates, hydrocarbons, flavonoids, terpenoids, vitamins, glycosides, etc. and is useful in the treatment of anasaraca, diseases of urinogenital tract, dropsy of chronic Bright's disease, hyperdipsia, vesical calculi, flatulence, diarrhea, dysentery, leucorrhoea, gonorrhea, asthma, blood diseases, gastric diseases, painful micturition, menorrhagea, etc. (Rastogi and Mehrotra, 1993; Annonymous, 2002; Sharma *et al*., 2002 and Nadkarni, 2007).

Previous phytochemical investigations of *H. schulli* led to the isolation of isoflavone glycoside, an alkaloid and small quantities of uncharacterized bases (Nikam *et al*., 2012). Nikam *et al*. (2012) also reported that *H. schulli* contains lupeol, stigmasterol, isoflavone glycoside, an alkaloid and small quantities of uncharacterized bases.

2.5. Therapeutic uses of *H. schulli*.

H.schulli (Syn. *Hygrophila auriculota* (K. Schum.) Heine, *Asterocantha longifolia* Nees) is a reputed ayurvedic herb known as lkshura, lkshugandha and Kokilaksha. All parts of plant are medicinally useful. The plant is sweet, sour, bitter, aphrodisiac and tonic. Root is diuretic, anti-inflammatory, used in ascites, vesical calculi, jaundice and dysentery. Leaves are used in jaundice, rheumatism and diseases of urinogenital tract. Seeds are rejuvenating, nervine tonic and useful in gonorrhea and renal disorders (Warrier *et al*., 1996).

The plant is also extensively used in folk medicine for body pain, impotency, jaundice, malaria, rheumatism, body swelling, tuberculous fistula and as aphrodisiac. Traditionally, the leaves are used as/in diuretic, jaundice, antibacterial, dropsy, rheumatism, anasaraca, diseases of urinogenital tract, leucor, sweet, sour, bitter, tonic, oleaginous, aphrodisiac, hypnotic, diarrhea, dysentery, urinary calculi, urinary discharge, anti inflammatory, joint pain, biliousness, eye disease, ascites, abdominal troubles, anemia, anuria, gleets, cough, demulcent, stomachic, lumbago, arthritis, gastric disorder and leucorrhoea (Patra *et al.*, 2009).

Hewawasam *et al.* (2003) tested the aqueous extract of *Asteracantha longifolia* for hepatoprotective activity against carbon tetrachloride- and paracetamol induced acute hepatotoxicity in mice. The plant exhibited significant hepatoprotective activity by reducing carbon tetrachloride- and paracetamol induced changes in liver enzymes. The plant extract may interfere with free radical formation, which may account for the hepatoprotective action.

Ahmed *et al.* (2001) reported the anti-tumor activity of seeds of *A. longifolia* against experimental hepato-carcinogenesis in rats. They also stated that the seeds significantly ameliorated the activities of antioxidant enzymes glutathione peroxidase and catalase in a dose dependant manner (Ahmed *et al*., 2001).

Mazumdar *et al.* (1997) reported that the petroleum ether extract of the roots of *H. spinosa* exhibited anti-tumour activity in *Ehrlich ascites* carcinoma and sarcoma-180 bearing mice. A hydroalcohlic extract of whole plant of *H. spinosa* at a dose of 300mg/kg body weight showed significant anti-tumour activity against 7, 12- dimethylbenz (a) anthracene (DMBA) induced mammary tumours in female rats comparable with tamoxifen as a standard drugs (Pattanayak *et al*., 2008).

Balasubramaniam *et al.* (2012) reported that the effect of *H. auriculata* on carbohydrate metabolizing enzymes in N-nitrosodiethylamine induced hepatocellular carcinoma in rats.

The methanolic extract of *H. auriculata* (200 mg/kg) produced significant decrease in hexokinase, phosphogluco-isomerase, aldolase, while increased glucose-6-phosphatase in the plasma and liver of carcinoma bearing rats. When

compared with the control, methanolic extract of *H. auriculata* was found to revert the altered carbohydrate metabolizing enzymes which is associated with biochemical changes of hepatomas to near normal in HCC bearing rats due to the presence of polyphenols and flavonoids (Balasubramanian and Premkumari ,2012).

Sunilkumar and Klausmuller (1999) screened 28 different plant species of Nepalese medicinal plants including seeds of *A. longifolia* used traditionally in indigenous system of medicine to treat inflammatory diseases for the inhibitory effect on lipid peroxidation and reported that the plant inhibited lipid peroxidation with an IC_{50} value of 20 µg/mL.

2.6. Chemical characterization of *H.schulli*

Analytical methods have been used to characterize compounds from herbal medicine, their products, and extracts. Thermal analysis, IR spectroscopy, GC-MS, SEM and X-ray diffraction are examples of methodologies used for characterisation of the extract have to be based on these compounds. When the active principle of the extract is not known, lead compounds are usually used for chemical characterization. The compounds may not be related to their activity, but they should be typically chemical markers of the extracts (Bauer and Tittel, 1996).

UV-Visible related to the spectroscopy of photons in the UV-visible region. A spectrum is obtained when the absorption of light is measured as a function of its frequency or wavelength. Molecules with electrons in delocalized aromatic systems often absorb light in the near-UV (150-400nm) or the visible (400-800nm) region (Schmid, 2001).

FT-IR is a preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis including plant extracts.

The discovery of X-rays in 1895 enabled scientists to probe crystalline structure at the atomic level. X-ray diffraction has been in use in two main areas, for the fingerprint characterization of crystalline materials and the determination of their structure. Each crystalline solid has its unique characteristic X-ray powder patternwhich may be used as a "fingerprint" for its identification. Once the material has been identified, X-ray crystallography may be used to determine its structure, i.e. how the atoms pack together in the crystalline state and what the interatomic distance and angle are etc. X-ray diffraction is one of the most important characterization tools used in solid state chemistry and materials science.

To explore the importance of any medicinal plant the initial step is to screen for its phytochemicals, as it gives a broad idea regarding the nature of compounds present in it. In GC-MS, the chromatogram obtained from the samples were analysed for the presence of active components present in the sample. The method is very simple, it takes only few minutes to complete and uses few solvents (Gherman *et al*., 2000).

The methanol extract of whole plants of *H. schulli* was subjected to repeated chromatographic separation and purification processes to isolate its chemical constituents. A total of three compounds were isolated which have been characterized as stigma sterol, lupeol and lup-20(29)-ene-3β,23- diol on the basis of 1H NMR spectral evidence and confirmed by comparing with published data, as well as co-TLC in case of stigma sterol and lupeol. This is the first report of lup-20(29)-ene-3β, 23-diol from this plant (Sufian *et al*., 2015).

2.7. Antioxidant activity of *H. schulli*.

Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids and tocopherols (Ali *et al.,* 2008). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Krings and Berger, 2001). Flavonoids and tannins as phenolic compounds and other plant phenolics are major group of compounds that act as primary antioxidants or free radical scavengers (Saxena *et al.,* 2012).

Majority of the diseases/disorders are mainly linked to oxidative stress due

to free radicals (Gutteridge, 1995). Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism (Tiwari, 2001).

The most common reactive oxygen species (ROS) include superoxide (O_2.) anion, hydrogen peroxide (H_2O_2), peroxyl (ROO-) radicals, reactive hydroxyl (OH.) radicals, nitric oxide (NO.) and peroxynitrite anion (ONOO.) (Yildrim *et al*. 2001). Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides (Buyukokuroglu *et al.,* 2001).

ROS play an important role in degenerative or pathological processes, such as aging, cancers, coronary heart diseases, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts (Huang *et al*., 2005), arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome.

ROS can cause lipid peroxidation in foods, leading to their deterioration. In addition, these ROS can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxidation. The peroxidation products and their secondary oxidation products such as malondialdehyde and 4-hydroxyinonenal can react with biological substrates such as protein, amines, and deoxyribonucleic acid (Gulcin *et al*., 2003).

As a result of this, much attention has been focused on the use of antioxidants, especially natural antioxidants to inhibit lipid peroxidation and to protect from damage due to free radicals. The term 'antioxidant' refers to the activity of numerous vitamins, minerals and phytochemicals which provide protection against the damage caused by ROS (Khilfi *et al*., 2006).

A great number of aromatic and other medicinal plants contain chemical compounds that exhibit antioxidant properties. Sources of natural antioxidants are primarily, plant phenolics that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt and Hudson, 1990; Mathew and Abraham, 2006).

Living organisms have antioxidant defence systems that protects against oxidative damage by removal or repair of damaged molecules(Sun *et al*.,1998) Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors (Gulcin *et al*., 2005).

Antioxidants are widely used in dietary supplements and have been investigated for the prevention of disease such as cancer, coronary heart disease and even altitude sickness Considerable laboratory evidence from chemical, cell culture and animal studies indicates that antioxidants may slow or possibly prevent the development of cancer (Antioxidant and Cancer prevention: Fact sheet).

Antioxidants can be classified into a number of different groups as enzymatic and non-enzymatic strategies. The enzymatic antioxidant involve superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, while non-enzymatic antioxidants include the vitamins A, C, and E, glutathione, and lipoic acid, mixed carotenoids, several bioflavonoids, antioxidant minerals (copper, zinc, manganese, and selenium), etc. Vitamin E inhibits the propagation of lipid peroxidation; the combination of vitamin C and vitamin E suppress the formation of hydroperoxide; metal complexing antioxidants such as penicillamine inhibit free radical formation in lipid peroxidation (Feher *et al.,* 1987).

The first large randomized trial on antioxidant and cancer risk was the Chinese cancer prevention study, published in 1993. This trial investigated the effect of a combination of beta-carotene, vitamin E and selenium on cancer in healthy Chinese men and women at high risk for gastro cancer. The study showed a combination of beta-carotene, vitamin E and selenium significantly reduced incidence of both gastric cancer and cancer overall (Blot *et al*., 1993).

In treatment of many diseases, antioxidant therapy has gained an immense importance. Current research is now directed towards finding naturally occurring antioxidants of plant origin. Antioxidants have been reported to prevent oxidative damage by free radicals and ROS, and may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free

radicals, chelating, catalytic metals, and also by acting as oxygen scavengers (Buyukokukuroglu *et al*., 2001; Shahidi and Wanasundara, 1992).

Several medicinal plants have been screened for potential antioxidant activity because of no side effects and economic viability (Auudy *et al*., 2003). Radical scavenging effects of *H. schulli* were found to be of quite high level (Doss *et al*., 2009). Hepatoprotective activity of *H.schulli* has been attributed to increased regeneration of hepatocytes and inhibitory effects on microsomal enzymes (Gurusamy *et al*., 2010).

Antioxidant activity of the extracts of varying concentrations ranging from 10- 10000 µg/ml was evaluated by various *in vitro* models. It was observed that the test compounds scavenged free radicals in concentration dependent manner in all the models (Parr and Bowell, 2000).

2.8. Antimicrobial activity of *H. schulli*

During the last two or three decades, rapid increase in the rate of infections, antibiotic resistance in microorganisms, side effects of synthetic antibiotics, together with advances in phytochemistry and identification of new bioactive compounds from plants which are effective against certain diseases, have renewed the popularity of herbal medicines (FAO, 1993; Babu and Subhasree, 2009).

The resistance of pathogenic microorganisms to currently known antibiotics is constantly increasing due to a broad use of antimicrobials in medicine, animal husbandry and agriculture. If no preventive measures are taken, such events will certainly increase with time, this will inevitably lead to the development of novel antibiotics with alternative therapeutic strategies is essential.

The multiple drug resistance of microorganisms is a global concern today in view of the emergence and persistent spread of resistant microbial strains throughout the world owing to their phenotypic plasticity (Kunin, 1993; Blondeau 1999).

Among many proposed strategies, a good understanding of systematic screening of traditional system of medicine offers the potential of developing potent broad spectrum antibiotics. These documents opens up an expanding

spectrum of applications and better understanding of diagnostic and therapeutic purposes to get effective antimicrobial agents.

Boily and Vampuyvelde (1986) screened anti-microbial property of ethanolic extract of leaves, stem, fruits and root of *H. auriculata* against *Staphylococcus aureus, Pseudomonas aeroginosa, Bacillus subtilis, Escherachia coli, Candida albicans* and *Mycobacterium smegmatis* and reported that the leaves exhibited active anti-microbial activity against *S. aureus, B. subtilis, C. albicans and M. smegmatis.*

Vlientick *et al.* (1995) screened anti-microbial property of ethanolic extract of leaves, stem, fruits and roots of *H. auricalata* against Staphylococcus aureus, *Pseudomonas aeroginosa, Escherichia coli, Candida albicans, Tricophyton mentagraphytes* and *Mycobacterium canis* and reported that the leaves exhibited active anti-microbial activity against *S. aureus, C. albicans, M. canis* and *T. mentagraphytes*, stem exhibited activity against *C. albicans, M. canis and T. mentagraphytes.*

The antibacterial activity of petroleum ether, chloroform, alcoholic and aqueous extracts of leaves of *H. spinosa* against *Escherachia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeroginosa* evaluated by disc-diffusion methods. The chloroform and alcoholic extract exhibited significant antibacterial activity, whereas the aqueous extract has moderate effect and petroleum ether extract was showed least action against the microorganisms (Patra *et al*., 2008).

III. MATERIALS AND METHODS

The materials used and methods adopted in the present study entitled **"Antioxidant and antimicrobial activities of *Hygrophila schulli* (Buch.-Ham.)"** is presented in the following headings.

3.1 .Collection and authentication of the plant

The fresh plant of *H. schulli* was collected from Kuttanad wetlands, Alappuzha (Dist.), Kerala (Fig.1). The plant material was identified and their authenticity was confirmed at the herbarium of Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu (BSI/SRC/5/23/2015/Tech-1422).

Fig.1. A view of *Hygrophila schulli* on its habitat

3.2 . Preparation of the plant powder

The fresh plant was cleaned thoroughly and dried at room temperature for 5-7 days in the shade. The dried samples were powdered using an electrical grinder (Fig.2). The powdered samples were stored in screw cap bottles until further analysis.

Fig. 2. Dried plant and powder

3.3. Preparation of extract

One hundred grams of powder from the whole dried plants of *H. schulli* was taken, to which 100 ml of different solvents (chloroform, ethanol, methanol and water) were added, mixed, and kept for four days. The contents were periodically shaken using an electric shaker. After four days, the contents were filtered through a Buchner funnel in a conical flask and it was further concentrated by evaporation by keeping the filtrate in a round-bottomed flask, till the solvent completely evaporated and the extract settled down to the bottom (Fig.3).

Fig.3. Various solvent extracts

3.4. Qualitative phytochemical analysis

Preliminary screening of all the extracts were done by colour tests adapting standard methods by Raman (2006).

1. Test for Alkaloids

Mayer's test: A fraction of extract was treated with Mayer's test reagent (1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml of water) and observed for the formation of cream colour precipitate.

Wagner's test: A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and observed for the formation of reddish brown colour precipitate.

Dragendroff's test: 1ml of the extract was added to 1ml of Dragendroff's reagent. Appearance of orange colour precipitation indicates the presence of alkaloids.

2. Test for Flavonoids

Alkaline reagent test: 1 ml of the extract was treated with aqueous NaOH and HCl. The formation of yellow orange colour indicates the presence of flavonoids.

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

3. Test for Sterols

Liebermann-Burchard test: Extract (1ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red colour.

Salkowski's test: When concentrated sulphuric acid was added to a chloroform solution to the extracts (10 mg of extract in 1 ml of chloroform), a reddish blue colour was produced in the chloroform layer and green fluorescence in acid layer, suggesting the presence of steroids.

4. Test for Phenols

Ferric chloride test: The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour

5. Test for Saponins

Foam test: To the extract, 20 ml of distilled water was added and agitated on a graduated cylinder for 15 minutes. The formation of about 1 cm layer of foam indicates the presence of saponins.

6. **Test for Tannin**s

Gelatin test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Test for Quinones

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

8. Test for Proteins

Ninhydrin test: A small quantity of extract solution was boiled with 0.2% solution of Ninhydrin. Blue colour indicates the presence of amino acids.

Biuret test: The extract was treated with equal volume of 40% Sodium hydroxide and two drops of 1% copper sulphate solution. Pink or purple colour indicates the presence of proteins.

9. Test for Carbohydrates

Molisch's test: To small quantities of solvent free methanolic extract, few drops of 1% α-naphthol in ethanol were added. Conc.H_2SO_4 was then added to sides of the test tubes. A brown purple ring formed at the junction of the two liquids indicates the presence of sugars.

Fehling's test: Small quantities of solvent free methanolic extract were separately dissolved in minimum amount of distilled water and filtered. To the filtrates equal volume of Fehling's solution were mixed in a test tube separately and heated for few minutes. Formation of brick red precipitate confirmed the presence of sugars.

3.5. Chemical characterization

The methanol extract of *H.schulli* was subjected to various spectral analyses for the identification of chemical nature of the active components present in the plant.

3.5.1. UV-Visible Spectral analysis

UV-Visible spectrum profile of the sample was detected with a Shimadzu, UV-1700 spectrophotometer at wavelength ranging from 200-800nm.

3.5.2. FT-IR Spectral analysis

For FT-IR measurements dried powder of plant sample was used. 10mg of the powder was encapsulated in 100mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of plant specimen was loaded in FT-IR spectroscope with a scan range of 400-4000cm^{-1} with a resolution of 4cm^{-1}. The peaks obtained were plotted as % transmittance in X-axis and wave number (1/cm) in Y- axis.

3.5.3. X-Ray Diffraction analysis

The X-Ray diffraction pattern of the powder samples were recorded on a PANanalytical X'PERT PRO X-ray diffractometer using Cu K-alpha radiation (λ=1.54060 Å). The particle characterization was done by measuring the crystallite size of the sample from the line broadening analyses using Debey-Schherer formula after accounting for instrumental broadening.

3.5.7. GC-MS analysis

Chromatographic analysis was carried out using thermo GC-Trace Ultra Ver: 5.0 GC-MS (Model Thermo MS DSQ II gas chromatograph). A fused-DB35-MS Capillary standard Non-polar Column Dimension (30mts, ID: 0.25 mm, FILM: 0.25μm) was used. The GC temperature program was as follows: initial temperature was 75 °C, held for 2 min, increased to 150 °C at a rate of 2 °C/min, then to 220 °C at a rate of 3 °C/min, and finally to 260 °C at a rate of 6 °C/min and held for 10 min. The split ratio was 1:12, injection temperature was 250 °C, transfer line temperature was 270 °C, and the mass spectrometer was operated at 70 eV in run time 29 min.

3.6. Antioxidant studies

3.6.1. DPPH free radical scavenging activity (Mensor *et al*., 2001)

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 10-40 μl/ml solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below:

Percent (%) inhibition of DPPH activity = 100- (A-B/A) x100

Where A = optical density of the blank and B = optical density of the sample.

3.6.2. Reducing power assay (Oyaizu, 1986)

Reaction mixtures were prepared by adding 2.5 ml of phosphate buffer (0.2 M, pH 6.6), 2.5 ml potassium ferricyanide (1%) and varying concentrations of extracts (10-40 µl/ml). After, the reaction mixtures were incubated at 50°C in water bath for 30 min, allowed to cool at room temperature (28°C), and 2.5 ml of 10% TCA (Trichloroaceticacid) were added to each reaction mixture, and then centrifuged at 2000 rpm for 10 min. The supernatant (2.5 ml) was separated in the test tube and added with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1.0%), and allowed to react for10 min at room temperature and the absorbance was measured at 700 nm. Ascorbic acid solution was used as standard.

3.7. Antimicrobial activity

3.7.1. Test organisms

Two Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) and four Gram-negative bacteria (*Escherichia coli, Salmonella typhi, Klebsiella spp., Proteus*) were used for antibacterial activity study, four fungi (*Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus* and *Trichoderma*) were used for antifungal activity.

3.7.2. Antibacterial activity

The antibacterial sensitivity assay was carried out by disc diffusion method of Bauer *et al*. (1966) and different solvent extracts of the plant leaves and roots were tested against the selected test bacterial strains. The test bacterial cultures were evenly spread over Mueller Hinton agar plates using a sterile cotton swab. The sterile discs (6 mm in diameter) were impregnated with extract solution and placed in the inoculated agar. The plates were thus incubated at 37°C for 24 to 48 hours. After incubation, the results were observed and the zones of inhibition thus developed were measured with the scale to the nearest in mm. Chloramphenicol (K-30µg/disc) was used as a positive control and respective solvents serves as a negative control. The experiment was done in triplicates and the mean values were presented. The extracts shown high antibacterial activity was

further subjected to disc diffusion assay at varying concentrations (10, 15, 20, 25 and 30 mg/ml) to check for the maximum activity at a definite concentration.

3.7.3. Antifungal activity

Antifungal activity was measured using disc diffusion method Ronald (1990). The fungal cultures to be tested were evenly spread over respective agar plates using a sterile cotton swab. Then, sterile paper discs (6mm diameter) impregnated with suitable extract was placed on agar. The activity was determined after 72 hours of incubation at 27oC. Nystatin (50 µg/disc) was used as a positive control and respective solvents as negative controls. Inhibition zones were determined after incubation at 27oC for 48 hours. All tests were done in triplicate and the mean values were presented.

Procedure

The pure cultures of different pathogens were grown overnight in sterile nutrient broth and incubated at 37°C for 24 hours. The 0.1ml of the culture was seeded on 25 ml of solidified nutrient agar and rose bengal plates for bacterial and fungal cultures, respectively. The wells were bored with 8mm borer in seeded agar, and then the particular concentrations of the extracts were added in each well. Soon after the plates were then kept at 10°C for 30min. After it normalized to room temperature plates were incubated at 37°C for 24hrs. After incubation period is completed, the zone of inhibition was measured and recorded.

IV. RESULTS

The results pertaining to the study **"Antioxidant and antimicrobial activities of *Hygrophila schulli* (Buch.-Ham.)"** are presented in the following headings.

4.1. Qualitative phytochemical analysis

The phytochemical constituents serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Investigations on secondary plant constituents have made phenomenal advance during the past few decades. Based on the above concept few analysis were done with various solvents used in the present study which are described below.

Results showed the presence of alkaloids, terpenoids, sterols, carbohydrates, saponins and flavonoids. Maximum intensity of the phytochemicals was observed in methanol extract. Table I showed the presence or absence of various phytochemicals. So, for further analysis Methanol Extract of *Hygrophila schulli* (MEHS) was used.

Table I : Qualitative analysis of the extracts of *Hygrophila schulli*

S. No.	Constituents	Test for constituents	Solvents				
			Methanol	Aqueous	Chloroform	Ethanol	Petroleum ether
1	Alkaloids	Mayer's	+	-	-	-	-
		Wagner's	+	-	-	+	-
		Dragendroff's	+	-	-	-	+
2	Flavonoids	Alkaline reagent	-	-	+	+	-
		Lead acetate test	+	+	+	-	+
3	Sterols	Libermann Burchard	+	+	-	-	-
		Salkowski	+	+	-	-	+
4	Phenols	Ferric chloride	-	+	-	-	-
		Lead acetate	+	-	-	-	-
5	Saponins	Foam test	-	-	-	-	+
6	Tannins	Gelatin test	-	-	+	-	-
7	Quinones	Alcoholic KOH	+	-	-	-	-
8	Proteins	Ninhydrin	-	-	+	-	-
		Biuret test	-	-	+	-	-
9	Carbohydrate	Molisch's test	-	-	-	-	+
		Fehling's test	-	-	-	-	+

+ Present - Absent

4.2. Analysis of bioactive components

4.2.1. UV-Visible spectral analysis

The UV-Visible spectrum profile of *H.schulli* was chosen at a wavelength of 200-800nm due to sharpness of the peaks and proper baseline. The profile showed the peaks at 664, 610, 532, 400, 280 and 220nm with the absorption of 0.585, 0.280, 0.302, 1.967, 4.00 and 4.00 respectively (Table II& Fig.4).

Table II: Absorption peaks of UV-Vis spectrum

SI. No	Wavelength	Absorbance
1	664	0.58
2	610	0.28
3	532	0.30
4	400	1.96
5	280	4.00
6	220	4.00

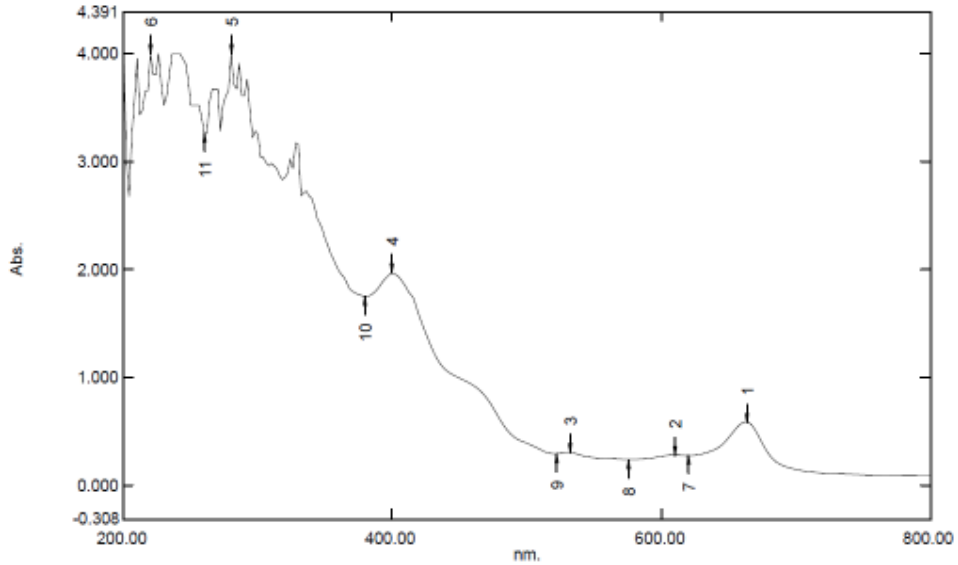


Fig. 4. UV-Visible spectrum of *Hygrophila schulli*

4.2.2. FT-IR analysis

The spectra showed various bands occurring at 2854.65, 3340.71, 2924.09, 1627.92, 1049.28 and 925.83 cm $^{-1}$ corresponding to the presence of C-H stretching, -OH group, C-H stretching, C=C group, C-O stretching and C-O stretching respectively (Table III & Fig. 5).

Table III: Peak values and functional groups of *Hygrophila schulli*

Peak value	Functional group
3340.71	Phenols
2924.09	Alkane
2854.65	Alkane
1627.92	Alkene
1049.28	Amine
925.83	Aromatic

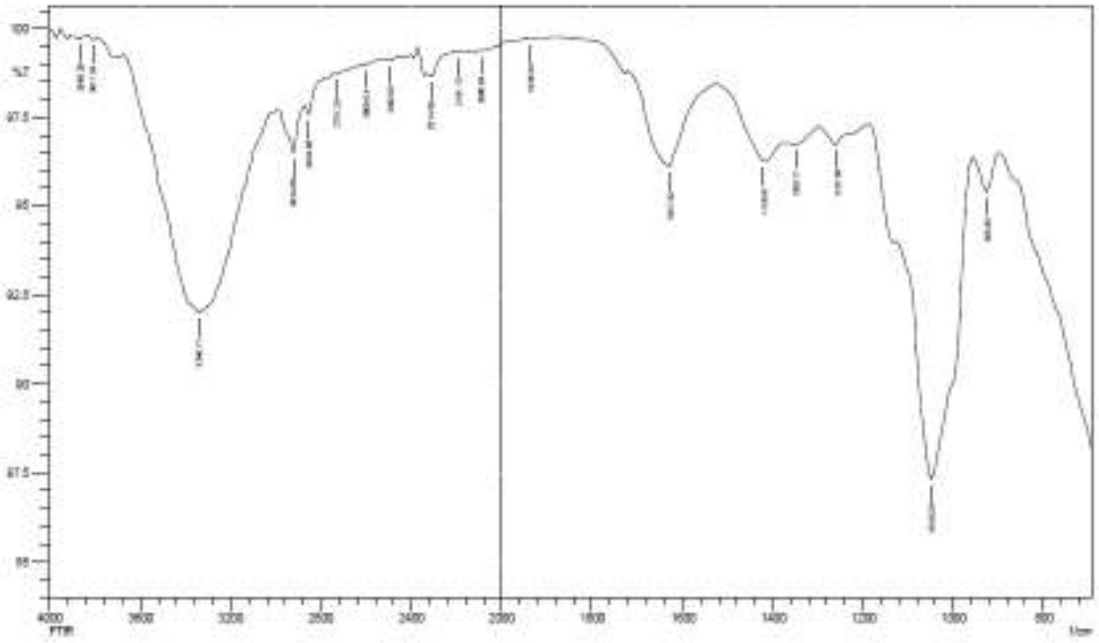


Fig.5. FT-IR spectrum of *Hygrophila schulli*

4.2.3. XRD- analysis

XRD is commonly used for the determination of crystal structure and identification of unknown crystalline materials (e.g. minerals, inorganic compounds) present in a sample. In the present study, nine prominent diffraction peaks were observed at 2θ positions of 14.9, 20.8, 24.51, 28.38, 30.16, 31.12, 40.60, 46.42 and 68.48(Table IV & Fig. 6).

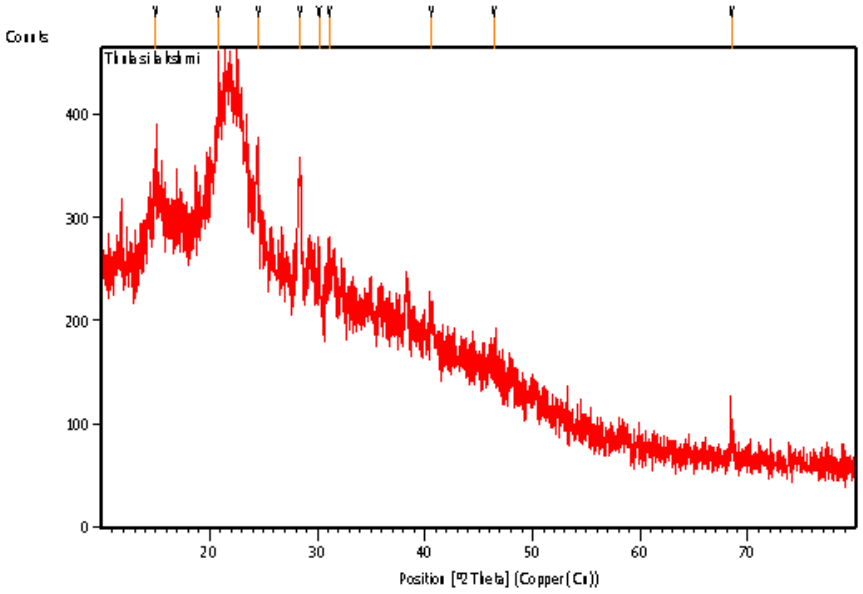


Fig. 6. XRD pattern of *Hygrophila schulli*

The Braggs reflections (78.19, 56.53, 70.79, 110.10, 42.41, 28.84, 20.46, 17.91 and 15.50) clearly indicated the presence of sets of lattice planes and further. The observed peak broadening and noise were probably indicating the presence of macromolecules present in the plant powder. The line broadening of the peaks are primarily due to small particle size.

Table IV: Peak list of the X-Ray diffractogram

Position [2θ]	Height [counts]	*FWHM Left [2θ]	d-spacing [A°]	Relative Intensity [%]
14.9985	78.19	0.2007	5.90698	71.01
20.8275	56.53	0.4015	4.26508	51.35
24.5187	70.79	0.2676	3.63072	64.29
28.3819	110.10	0.2676	3.14470	100.00
30.1634	42.41	0.2007	2.96290	38.52
31.1223	28.84	0.6691	2.87377	26.19
40.6095	20.46	0.8029	2.22164	18.59
46.4293	17.91	0.8029	1.95582	16.26
68.4812	15.50	0.2676	1.37014	14.08

*FWHM Left: Full Width at Half Maximum left

4.2.4. GC-MS analysis

The results pertaining to the GC-MS analysis leads to the identification of fifty pharmacologically important compounds from the methanolic extract of *H. schulli* with nine major peaks at retention times 12.16, 24.45, 26.39, 28.35, 28.74, 28.96, 38.81, 46.13 and 46.98 minutes (Fig. 7).

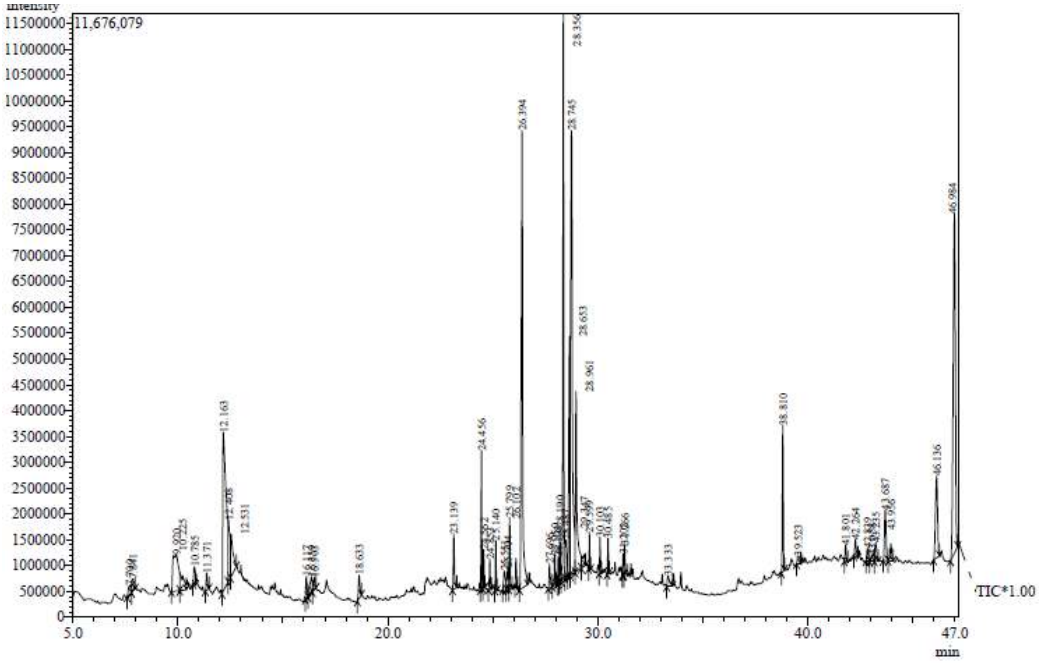


Fig.7. GC-MS profile of *Hygrophila schulli*

The major components are octadecatrienoic acid, stigmasterol, hexadecanoic acid, phytol, octadecanoic acid and nonanoic acid (Table V).

Table V: Major bioactive compounds of *Hygrophila schulli*

S. No	RT	Compound name	Molecular formula	Area %
1	28.74	9,12,15-Octodecatrienoic acid	$C_{18}H_{30}O2$	14.27
2	46.98	Stigmasterol	$C_{29}H_{48}O$	12.92
3	26.39	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	11.48
4	12.16	2-Furancarboxaldehyde	$C_6H_6O_3$	9.03
5	28.35	Phytol	$C_{20}H_{40}O$	8.60
6	28.65	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	4.99
7	28.96	Octadecanoic acid	$C_{18}H_{26}O_2$	4.43
8	9.92	Cyclopentanone	$C_7H_{14}N_2$	3.47
9	48.13	ERGOST-5-EN-3-01,(3.Beta.,24R)	$C_{28}H_{48}O$	2.91
10	38.81	2,6,10,14,18,22-Tetracosahexaene	$C_{30}H_5O$	2.34
11	12.53	Nonanoic acid	$C_9H_{18}O_2$	1.70

12	24.45	2,6,10-Trimethyl,14-Ethylene-	$C_{20}H_{38}$	1.67
13	43.68	Cholestrol	$C_{27}H_{46}O$	1.40
14	10.22	1,2,6-Trimethyl-hexane	$C_9H_{20}O_3$	1.05
15	28.48	Octadecanoic acid, Methyl ester	$C_{19}H_{38}O_2$	1.03
16	23.13	Tetradecanoic acid	$C_{14}H_{28}O_2$	1.00
17	25.79	Hexadecanoic acid ,methyl ester	$C_{17}H_{34}O_2$	0.96
18	28.19	9,12,15-Octadecatrienoic acid	$C_{19}H_{32}O_2$	0.80
19	25.14	3,7,11,15-Tetramethyl-2-hexadecen	$C_{20}H_{40}O$	0.61
20	25.55	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	0.60

The mass spectrum of MEHS at retention time 12.16min showed two characteristic (M-28), (M-29) peaks, m/e 126 and 97 indicating the presence of –C=O groups.

The mass spectrum of MEHS at retention time 24.45min showed four characteristic (M-13), (M-14), (M-27) and (M-28) peaks, m/e 123, 95, 82 and 68 indicating the presence of –CH, -CH_2 and –C=O groups.

The mass spectrum of MEHS at retention time 26.39min showed two characteristic (M-13) and (M-17) peaks, m/e 73 and 60 indicating the presence of –CH and OH groups.

The mass spectrum of MEHS at retention time 28.35min showed two characteristic (M-14), (M-16) peaks, m/e 71 and 57 indicating the presence of –CH_2 and –CH_3 groups.

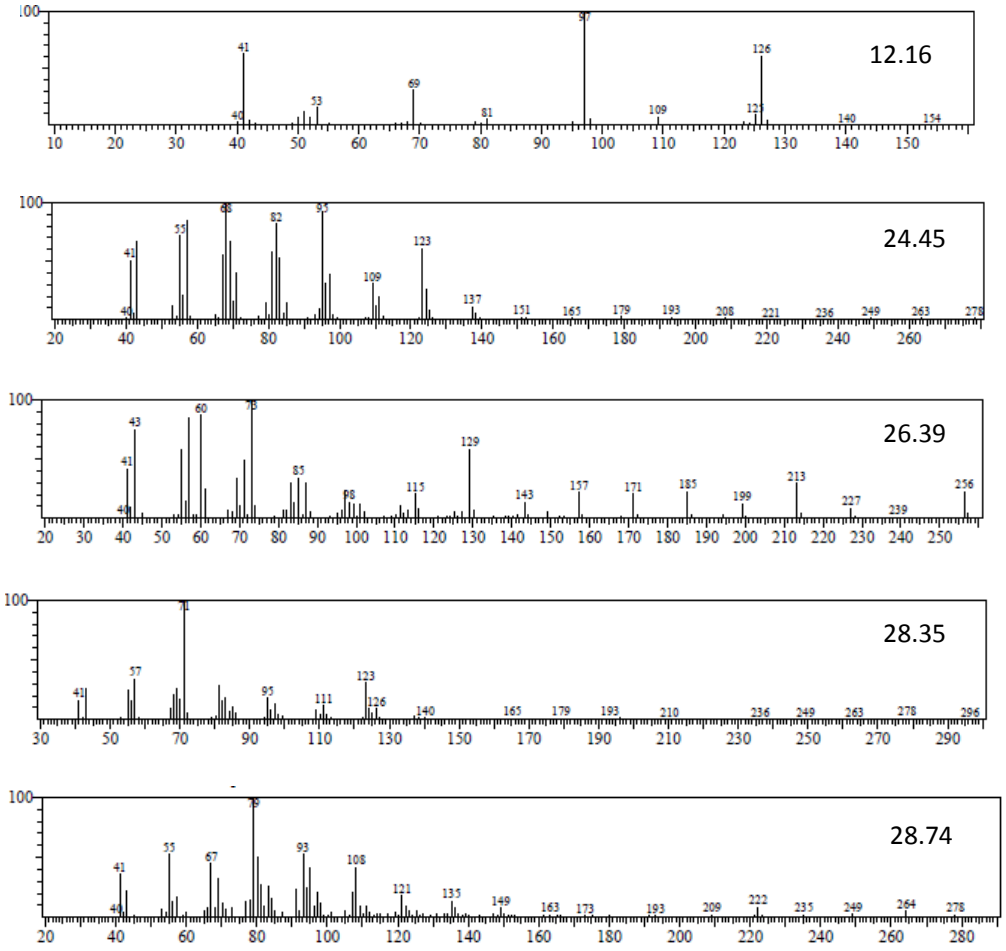
The mass spectrum of MEHS at retention time 28.74min showed three characteristic (M-14), (M-14) and (M-15) peaks, m/e 103, 93 and 79 indicating the presence of –CH_2 and –CH_3 groups.

The mass spectrum of MEHS at retention time 28.96 min showed two characteristic (M-14) and (M-16) peaks, m/e 73 and 53 indicating the presence of $–CH_2$ and $–CH_3$ groups.

The mass spectrum of MEHS at retention time 38.81min showed one characteristic (M-28) peak, m/e 81 indicating the presence of –C=O group.

The mass spectrum of MEHS at retention time 46.13min showed two characteristic (M-14) and (M-14) peaks, m/e 95 and 57 indicating the presence of $–CH_2$ groups.

The mass spectrum of MEHS at retention time 46.98 min showed four characteristic (M-14), (M-14), (M-14) and (M-14) peaks, m/e 97, 83, 69 and 55 indicating the presence of $–CH_2$ groups.



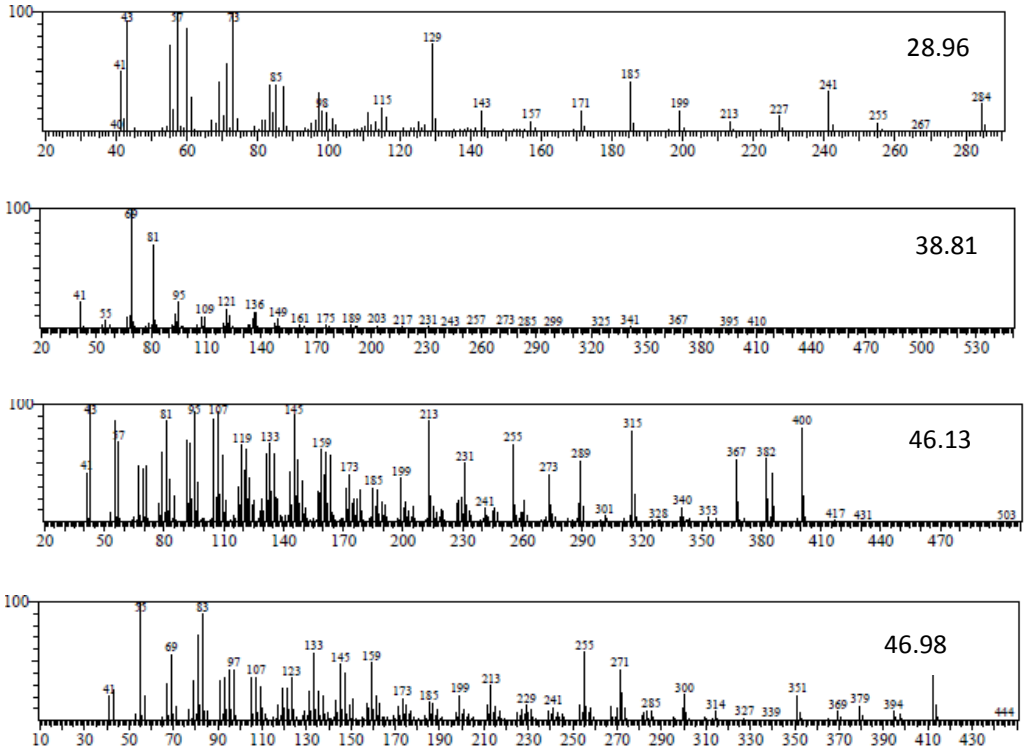


Fig.8. Mass spectrum of the compounds present in MEHS at different retention times.

4.3. Antioxidant activity

4.3.1. DPPH scavenging activity

The results of the assay are expressed in scavenging activity of DPPH free radical expressed in percentage. The analysis of data showed that the radical scavenging activity of the extract of *H. schulli* increased with increase in concentration of the extract (Table VI, Fig. 9).

Table VI: DPPH radical scavenging activity of *Hygrophila schulli*.

S. No.	Conc. of MEHS (mg/ml)	Scavenging Activity (%)
1	10	26
2	20	30
3	30	34
4	40	49
5	50	60

From the analysis, it was concluded that the scavenging effect of MEHS caused fifty percent inhibition (IC_{50}) was found to be at 41mg/ml (Fig.9).

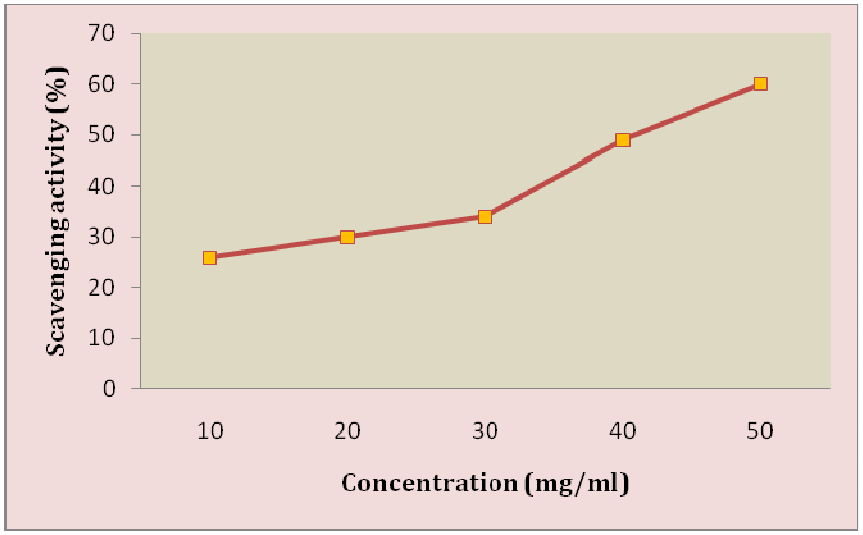


Fig.9. Scavenging activity of *Hygrophila schulli* against DPPH

4.3.2. Reducing power activity

The reducing power of different concentration of *H. schulli* was found to be remarkable and the absorbance of each concentration was found to rise as the

concentration gradually increased. Maximum absorbance was observed in 50mg/ml as shown in the Table VII & Figure 10.

Table VII: Reducing power assay of *Hygrophila schulli*.

S. No.	Conc. of MEPL (mg/ml)	Absorbance
		Methanol extract
1	10	0.29 ± 0.005
2	20	0.31± 0.15
3	30	0.32±0.017
4	40	0.38±0.005
5	50	0.39±0.01
6	Std	0.23 ± 0.02

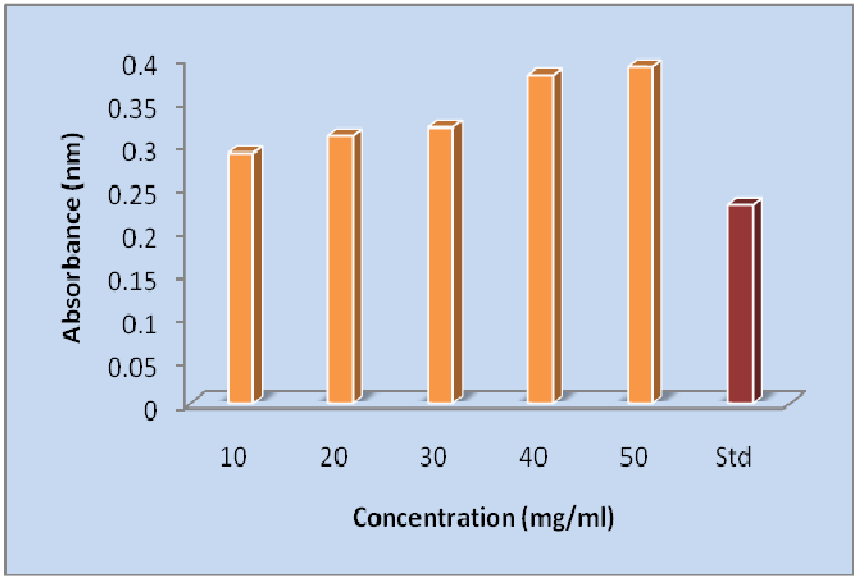


Fig.10. Reducing power ability of *Hygrophila schulli*

4.4. Antimicrobial activity of *Hygrophila schulli*.

4.4.1. Antibacterial activity

The different concentrations of MEHS showed promising antibacterial activity against all the bacteria tested. The zone of inhibition increased with an increase in the concentration of the extracts in the well. This showed the concentration dependent activity (Table VIII).

The different concentrations showed maximum activity against *Bacillus subtilis*, moderate activity against *Klebsilla*, but mild activity against *Salmonella typhi* (Fig.11).

Table VIII: Antibacterial activity of the methanol extracts of *H. schulli*

Test organisms	Zone of inhibition in mm					Control *
	10 (µl)	15 (µl)	20 (µl)	25 (µl)	30 (µl)	
Bacillus subtilis	10.0 ± 0.57	12.3 ± 1.15	13.3 ± 0.57	13.3 ± 1.0	16.0 ±1.0	21.6 ± 1.15
Staphylococcus aureus	10.0 ± 1.0	11.6 ± 0.57	11.6 ± 1.52	12.0 ± 1.73	16.0 ±1.0	20.0 ± 1.0
Escherchia coli	11.3 ±1.52	12.0 ± 1.0	12.0 ± 1.0	14.0 ± 1.73	15.3 ± 0.57	20.3 ± 1.52
Klebsilla sp.	9.0 ± 1.0	9.33 ± 0.57	10.3 ±1.52	15.0 ± 5	16.0 ± 1.0	22.0 ± 1.73
Salmonella typhi	7.30 ± 1.52	8.0 ± 1.0	11.3 ±1.15	11.6 ± 0.57	12.6 ± 0.57	21.3 ± 2.30
Proteus sp.	12.0 ± 2	13.0 ± 1.0	13.0 ± 1.0	13.3 ±1.0	16.66 ±1.52	20.6 ± 2.08

*** (Chloramphenicol, C^{30})**

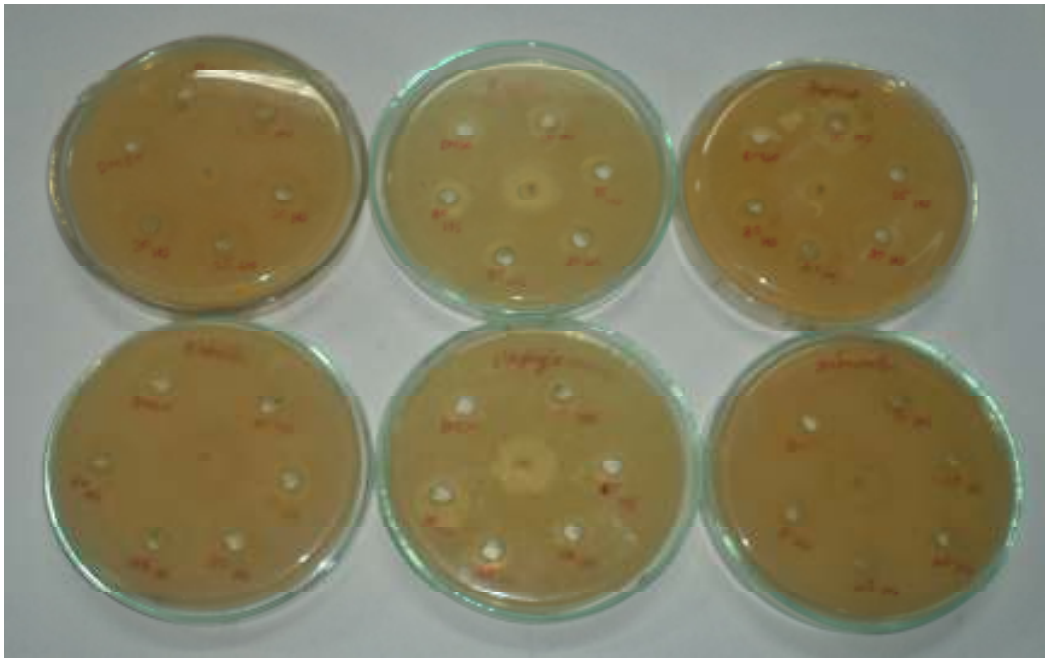


Fig. 11. Antibacterial activity of *Hygrophila schulli*

4.4.2. Antifungal activity

Similarly, the results demonstrated that all the test samples have potent antifungal activity against different *Aspergillus* species and *Trichoderma* tested here. The MEHS was significantly inhibited the growth of all the *Aspergillus* species and *Trichoderma* species (Table IX &Fig. 12).

Table IX: Antifungal activity of the methanolic extracts of *H. schulli*

Test organisms	Zone of inhibition in mm				Control (Nystatin)
	10 (µl)	15 (µl)	20 (µl)	25 (µl)	
Aspergillius fumigatus	19 ± 1.0	17± 1.0	14 ± 1.0	18 ± 1.0	27.66±3.21
Aspergillius niger	15.66 ±1.52	18 ± 1.0	13 ± 2.64	20 ± 1.27	29.33±1.52
Aspergillius flavus	15±1.0	14±1.0	19.66±1.52	14±1.0	25.33±1.52
Trichoderma	17 ± 1.0	15 ± 1.0	19±1.0	16.66 ±2.51	25.33±4

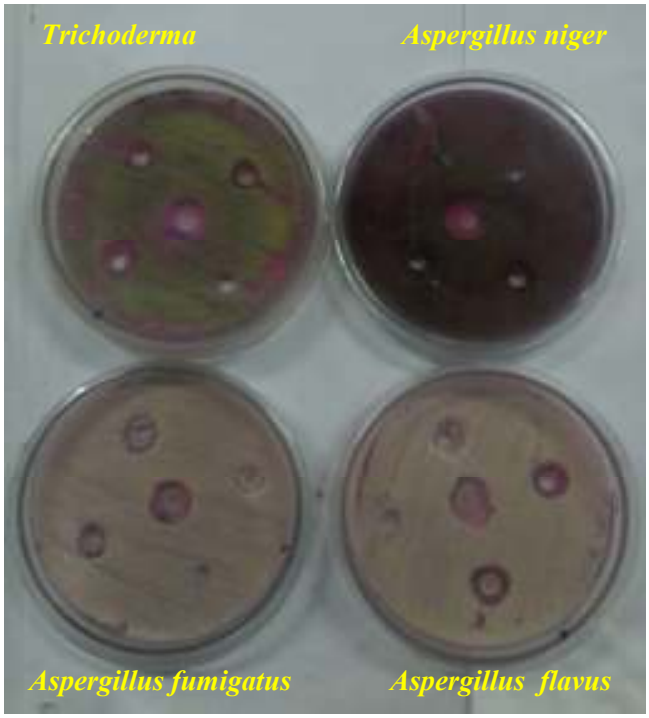


Fig.12.Antifungal activity of *Hygrophila schulli*

V. DISCUSSION

Plants have a great potential for producing new drugs of great benefit to mankind. Nowadays a renewed interest in traditional medicine is observed and there has been an increasing demand for more and more drugs from plant sources. This revival of interest in plant - derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs many of which have adverse side effects (Prabhakaran *et al*., 2010).

Today natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action (Charles *et al*., 2011). During the last two or three decades, advances in phytochemistry and identification of new bioactive compounds from plants which are effective against certain diseases, have renewed the popularity of herbal medicines (Babu and Subhasree, 2009). Hence, screening medicinal plants for promising biological activities in order to discover novel drug candidate is a necessity.

The demand for plant based medicines, health products, pharmaceuticals, food supplements and cosmetics are increasing in both developing and developed countries, due to the growing recognition that the natural products are non-toxic, have less side effects and easily available at affordable prices. India has lot of potential for producing world class herbal medicines. The Indian herbal Industry is on a roll and poised to grow in the coming years owing to its high demands for herbal products.

The modern medicine has evolved from folk medicine and traditional system only after thorough chemical and pharmaceutical screening. The use of synthetic compounds led to a decline in the use of plants in modern medicine however, synthetic medicine can cause side effects and as a result of people are more favourable to use natural compounds obtained from plants (Sunita *et al*., 2008).

Since the phyto-constituents and volatiles of medicinal herbs have created renewed demand in their use by the public, explorations of health benefits and antioxidant potential of these metabolites in the prevention of problems raised due

to oxidative stress is needed. Flavonoids and tannins as phenolic compounds and other plant phenolics are major group of compounds that act as primary antioxidants or free radical scavengers (Saxena *et al.,* 2012).

Medicinal plants therapeutic value can be evaluated on the basis of presence of chemical substances producing definite physiological action on human body involved in the defense mechanism of plants called phytochemicals. These photochemical are of different categories like Tannins, Cardiac glycosides, Phenolics, Flavanoids, Alkaloids, Anthocyanins, Essential oils, Terpenoids etc which got extracted to organic and aqueous phases according to their polarity while making extracts of different medicinal plants.

In the present investigation, preliminary phytochemical analysis indicated the presence of flavonoids, alkaloids, saponins, tannins, phenols, carbohydrates and triterpenoids in MEHS. Out of the five extracts analysed, the methanolic extract showed the maximum number of phytochemicals. When compared to aqueous, chloroform, petroleum ether and ethanol extracts, the methanolic extract showed high intensity. The ethanolic extract showed the least number of phytochemicals. Alkaloids were absent in the aqueous extract but present in all the other four extracts. This can be attributed to the relative insolubility of alkaloids in water as compared to organic solvents.

In general, plants with a high phenolic content showed high antioxidant activity. Therefore, there is a positive correlation between total phenolic compound and antioxidant activity (Chanda & Dave, 2009). However, some researcher reported no correlation between phenolic content and antioxidant capacity (Yu *et al*., 2002).

Souri *et al.* (2008) found that there was no significant correlation between antioxidant activity and phenolic content of the studied plants. However, nonphenolic components in plants such as trace elements can reduce the antioxidant activity of the phenolic compounds (Vinson *et al*., 1998).

The phenols could show activity synergistically with non-phenolic compounds. For this reason, phenolic compounds would not be the only ones responsible for the antioxidant activity (Onyeneho & Hettiarachy, 1992).

The high total phenolic concentration in some plant extracts may be due to the presence of saponin (Grover *et al*., 2001), amino acid (Uchikoba *et al.,* 1998) and triterpenoids (Shih *et al*., 2006). Because of these, phenolic concentrations of plant are not always good indicators of antioxidant capacity.

Biochemical analysis showed the presence of alkaloids, flavonoids and tannins in both the extracts of *Cymbopogon citratus* and *Asparagus recemosus*, while the presence of steroids was found only in the aqueous extract. Ethanolic and aqueous extracts of *C.citratus* and *A.racemosus* were isolated and phytochemical analysis was performed. It has been observed that most of the secondary metabolites were identified in the polar extracts (Jyothi *et al*., 2004). Flavonoids are polyphenolic compounds that play an important role in balancing lipid oxidations, and are associated with antioxidant activity (Yen *et al*., 1993).

According to Tiwari *et al*. (2011), Phytochemical concentration and antioxidant potential in plant extracts are affected by factors such as parts of plant, types of solvents, method of extraction and variety in plant material. Gopinath *et al*. (2013) observed the absence of steroids in both aqueous as well as ethanolic extracts of Cymbopogon citrates and presence of alkaloids, flavonoids and tannins in both the polar solvents. Phytochemical analysis by Jayshree *et al*. (2013) revealed the presence of various active phytochemical constituents viz. alkaloids, flavonoids, tannins in methanolic extract whereas steroids were absent in the same.

Nagamani *et al.* (2012) in their study showed the absence of alkaloids, flavanoids, tannins and presence of steroids in ethanolic extract while all the phytochemical contituents were present in aqueous extract. Phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens.

A knowledge of the chemical constituents of plant is desirable for the synthesis of complex chemical substances and for discovering the actual significance of folklore medicine.

In recent years secondary plant metabolites have been extensively investigated as a source of medicinal agents (Okoli *et al*., 2009). In modern complex therapy and recovery, natural and chemical compounds are used (Porres *et al*., 2004).

Medicinal and healing properties of herbs are closely related to their chemical components which are classified into some major groups like alkaloids, acids, essential oils, steroids, saponins, tannins etc. and getting this into herbal remedy depends upon the solubility of these compounds in various solvents (Al-Daihan *et al*., 2013).

Spectroscopic techniques are based on interaction between electromagnetic radiation and molecules of the sample, and are very attractive tools due to simplicity, fast and easy mode of operation. Electromagentic spectrum is a classification of photons with various energies in to different spectral regions.

UV-Visible spectroscopy is used when high energy photons are absorbed by the molecules of the sample which cause electronic excitation. Visible wavelengths cover a range from 400-800nm. Here the UV absorption spectrum of *H.schulli* extract was recorded at wavelength from 200-800nm. The profile showed six peaks, two between 200-300nm, one minor at 300-400nm and three of them between 400-600nm.

An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum (Eltahir *et al*., 2013). In the present study, the FT-IR spectral studies of *H.schulli* revealed the presence of mainly hydroxyl groups, imply that the presence of phenolic compounds.

Similar results were reported by Patra *et al*. (2010) who reported the FT-IR analysis of leaves of *H.schulli* showed the presence of hydroxyl groups in the chloroform extract. Packialakshmi and Naziya (2014) reported various functional groups like halogen, alkanes, carboxylic acid and aldehydes. Starlin *et al*. (2012) indicated the presence of amino acids, amines, amides, carboxylic acids, carbonyl compounds, organic hydrocarbons and halogens in the ethanolic extract of *Tylophora pauciflora*.

Powder diffraction data are a source of quantitative structural information when coupled with recently developed computational methods. In the present investigation, nine diffraction peaks were observed. The Braggs reflections clearly indicated the presence of sets of lattice planes and further. The observed peak broadening and noise were probably indicating the presence of macromolecules present in the plant powder. The line broadening of the peaks are primarily due to small particle size.

GC-MS analysis have been applied by many researchers to identify the possible bioactive components present in the plant extracts and herbal preparations, which might be useful for the identification of lead compounds for the development of new pharmaceutical drugs. It has been used in a number of occasions for the analysis of bioactive components in plants.

In the present study, *H.schulli* showed nine major peaks comprising fifty significant compounds by GC-MS analysis. The most important compounds identified were octadecatrienoic acid, stigmasterol, hexadecanoic acid, phytol, octadecanoic acid and nonanoic acid.

GC-MS studies have reported that the diethyl ether extract of the whole plant of *H. schulli* contains various phytochemical components. A particular peak found at RT 120 with a peak area of 100% showed the presence of Ellipticine. This compound is an alkaloid with a molecular formula of $C_{17}H_{14}N_2$ and molecular weight is 246. 31. Alkaloids play some metabolic role and control development in living system. Ellipticine shows antimicrobial activities (Sunita *et al*., 2008).

In another study, the peak at RT 104 with a peak area of 45% corresponds to the compound is 2-Furan carboxaldehyde hydroxyl methyl. The molecular formula and a molecular weight of this compound are $C_6H_6O_3$ and 126.12 respectively. 2-Furan carboxaldehyde hydroxyl methyl compound belongs to aldehyde group. It shows antimicrobial activity and preservative (Kumar *et al*., 2010).

The peak at RT 280 and peak area 25% is Queretin which is a flavonoid compound. This flavonoid is known to be most of medicinal activities like antidiabetic, anti-inflammatory, antidermatic, antileukemic and anticancer activities. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable (Gerige *et al*., 2007).

The hydroxyl group of compound 2-hydroxycyclo pentadecane was observed at peak at RT 162 with a peak area of 18% also reported to show antimicrobial activities. The peak at RT 309 with peak area 10% was 2, 3 dihydro benzofuran which has methylene group. This compound has less antimicrobial activities.

The intensity of the modern life style, the stress, the malnutrition, the low food quality, and the interaction of the exogenous and endogenous toxic factors and agents are among the cumulative causes of premature degradation of the human organ systems: central nervous, cardiovascular, endocrine and reproductive (Tavarajah *et al*., 2011). As a result, there are frequent liver diseases, ischemic heart diseases, diabetes, and urinary system disorders, accompanied by the depreciation of the immune function of the body.

The modern medication pleads for the introduction in treatment and recovery protocols of preparations capable of stimulating the body's protection capacity in different pathological states (Thavarajah *et al*., 2011).

These toxic factors are a direct consequence of the complex of phenomena collectively called "oxidative stress" defined as an exaggerated production of free radicals in the human body, accompanied by a decrease of antioxidant agents (Gohil *et al*., 2002; Ilieva *et al.*, 2013). A possible solution for this problem is to use natural antioxidants that neutralize free radicals and protect human body cells.

The antioxidants represent a group of compounds synthesized by the body, which can also be found in different natural food products (Esposito *et al.,* 2002). They act in synchrony with the human body, consuming free radicals and maintaining its health. The role of antioxidants consists in annihilating free radical activity in the human body, which are produced uninterrupted and are serving as catalysts for the metabolic processes (Amarowicz *et al*., 2010).

The oxidative stress represents a perturbation of the redox homeostasis or the totality of the oxidative deteriorations produced by an overproduction of reactive species (RS) or a decrease of the antioxidant (AO) system capacity at cell level or at body level.

Therefore, there is a deregulation of the pro-oxidant / antioxidant balance in favour of pro oxidants (Amarowicz *et al*., 2009; Butnariu *et al*., 2013). Oxide radicals have two categories of effects on the body, as follows: the beneficial effects, acting as bactericides within phagocytosis, stimulation of the activity of lymphocytes, control of vascular tonus, stimulation of the growth and proliferation of cells, stimulation of erythropoietin secretion and also negative effects such as destruction/deterioration of cell structure, cell malignant alteration and cell ageing (Wang *et al.,* 2006).

Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reaction can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves. An antioxidant is a molecule that inhibits the oxidation of other molecules.

Antioxidants can protect the cell from oxidative stress and it is the improper balance between ROS and antioxidants that deregulates the cellular functions leading to cancer (Bandyopadhyay *et al.,* 1999).

Different types of extracts are prepared from different parts of the medicinal plants are the source of these antioxidants. The antioxidants are generally dissolved in organic or aqueous compounds used while making extracts

from different parts of different medicinal plants thus becoming the good source of antioxidants which can be tested on different cancerous cell lines and later identified as proper chemical compounds.

Natural products have the potential to be developed into new drugs for the treatment of various diseases (Chen *et al*., 2009). Current research is now directed towards natural antioxidants originated from plants due to safe therapeutics (Lobo *et al*., 2010). It is believed that medicinal plants are a potential source of reactive oxygen species scavenger molecules (Anandjiwala *et al*., 2008).

The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability (Auudy *et al*., 2003). Plant extract could be utilized as a source of nutritional phenolics (Kuate *et al*., 2011). There is a growing interest in natural anti-oxidants present in medicinal and food plants that might attenuate oxidative stress (Silva *et al*., 2007).

Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic (Miller, 1996). They were also suggested to be potential iron chelators (Boyer *et al*., 1988; Havsteen, 1983).

Antioxidants may work either alone, or in association with each other against different types of free radicals. The different plant extracts will have different modes of action for curing diseases and in mixture form may exhibit enhanced activity than that of individual plants, which is known as 'synergistic action'. A particular principle in the pure form may have only a fraction of the pharmacological activity than it has in its plant matrix. This highlights the importance of using the plant as a whole for treating a disease (Arun *et al*., 2007).

DPPH is a stable free radical, which has been widely used in phyto-medicine for the assessment of scavenging activities of bioactive fractions. The antioxidant effect is proportional to the disappearance of the purple colour of DPPH in test samples. Thus antioxidant molecules can quench DPPH free radicals

by providing hydrogen atom or by electron donation and a colourless stable molecule 2, 2- di-phenyl -1 - hydrazine is formed and as a result of which the absorbance (at 517 nm) of the solution is decreased (Seal, 2012).

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability is generally associated with the presence of reductones which breaks the free radical chain by donating a hydrogen atom (Subhasini *et al.*, 2011).

In the present study, the methanol extract of *H.schulli* exhibited antioxidant and radical scavenging activities by inhibiting DPPH radical. Also in reducing power assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound and showed excellent reducing power ability. These effects of the extract could be attributed to the presence of bioactives present in the methanolic extract.

These results are in accordance with the findings of Olorunnisola *et al*. (2012) who evaluated the antioxidant activity of acetone and ethanolic leaves extracts of *Hippobromus pauciflorus* (L.F.) Radlk indicate that the extracts possess antioxidant properties and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants and could be used in the treatment and management of free-radical mediated diseases.

Also Faravani *et al*. (2009) and Susanti *et al*. (2009) reported the ability of *Melastoma malabathricum* extracts to scavenge the DPPH free radicals. Also Zakaria *et al*. (2011) determined that the aqueous and methanol extracts of *M. malabathricum* leaves had high antioxidant activity in the superoxide and DPPH scavenging assays and they recorded that the antiproliferative effects of *M. malabathricum* could be attributed to its high content of phenolic compounds.

In another study, the evaluation of anti-radical properties of nine wild edible plants was performed using DPPH radical scavenging assay. The 50% inhibition of DPPH radical (IC_{50}) by the different plant materials was determined, a lower value would reflect greater antioxidant activity of the sample. The highest

radical scavenging activity was shown by the aqueous methanol extract of *Gentiana pedicellata* (IC_{50} = 0.23 ± 0.0007 mg dry material), due to the hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary component as a radical scavenger (Seal, 2012).

The *in vitro* antioxidant activity, chelating ability and total phenolic content of the aqueous seed extract of *Tetracarpidium conophorum* have been reported (Olabinri *et al*., 2010). The *in vitro* antioxidant activity of the stem bark of *Parkia biglobosa* has been studied (Molliogo-Kone *et al*., 2009).

Diseases play an important role in a population. Infectious or otherwise, they greatly influence overall population growth. Infectious or communicable diseases are considered to be one of the leading causes of mortality, especially in developing countries.

The epidemicity of a disease is often caused by pathogens. Much research has been dedicated to finding solutions to halt the spread of disease-causing organisms, and has led to the discovery of antibiotics such as penicillin, erythromycin and Chloramphenicol (Tawiah *et al*., 2012). However, the rapid evolution of resistant pathogenic infections has become a great concern, because these antibiotics have become less effective (Cowan *et al*., 1999; Mothana *et al*., 2010). Therefore, the demand for new and alternative medicines has exponentially increased in recent years.

Bacterial diseases are a major health problem because they are responsible of numerous deaths per day worldwide. The world health organization (2002) and UNAIDS (2007) reported that between 14 and 17 million people die each year to the infectious diseases.

Infectious diseases cause enormous mortality in developing countries because of the extreme poverty of the people compared to developed countries. This situation is aggravated by the lack of suitable vaccine, inaccessibility and/or lack of antibiotics and the emergence of antibiotic-resistant strains.

Historically, the researchers made many efforts to discover new antibacterial compounds. One of important discovery source of these antibacterial drugs is medicinal plants throughout folk medicines. In addition it is known that

contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Iwu *et al.,* 1999).

In Burkina Faso, *Acanthaceae* is one of the greatest families most used in traditional medicine. *Hygrophila auriculata* (Schumach.) Heine, *Nelsonia canescens* (Lam) Spreng and *Peristrophe bicalyculata* (Retz.) Nees are three medicinal plants of this family very used traditionally to treat various infectious diseases such as diarrheal diseases, cholera, typhoid fever and tuberculosis.

In the present study, the antibacterial activity of the test organisms against MEHS extracts on the agar plates varied for different concentrations. The zone of inhibition increased with an increase in the concentration of the extracts in the well. The different concentrations showed maximum activity against *Bacillus subtilis*, moderate activity against *Klebsila*, but mild activity against *salmonella typhi.*

Previous antibacterial activities showed that leaves extract of *Hygrophila auriculata* for the period from September to October exhibited a good antibacterial activity on *Escherchia coli* (NCIM NO 2341)*, Staphylococcus aureus* (NCIM NO 2654)*, Bacilus subtilis* (NCIM NO 2195) and *Pseudomonas aeruginosa* (NCIM NO 2914) (Patra *et al.,* 2009).

Various antibacterial investigations were carried out on the crude extract obtained from leaves of *Asparagus recemosus* and *Cymbopogon citrtatus* for the screening of antimicrobial potential (Al-Daihan *et al*., 2013). Sankarnarayan *et al* (2011) reported that ethanolic extract of *A. recemosus* showed the zone of inhibition against *E. coli* and *Staphylococcus aureus* ranging from 16-24 mm and zone of inhibition with chloroform extract against same organism ranging from 16-20 mm.

Sinha and Biswas (2011) examined the methanolic extract of *Asparagus recemosus* for the antibacterial activity against isolated human pathogens and reported the zone of inhibition ranging from 3-15mm, hence he concluded that the methanolic extract of *A. recemosus* showed the maximum activity against three pathogens viz. *E.coli, Proteus vulgaris, Staphylococcus aureus.*

Rajendran *et al*. (2012) reported that *Asparagus recemosus* showed moderate inhibition activity with the zone range from 1-10 mm. Maximum zone of inhibition was observed with ethanolic extract against *E.coli* (10 mm) and minimum zone of inhibition with aqueous extract against *P.vulgaris* (1mm).Against all the tested bacterial strains, ethanolic extracts of *A.recemosus* showing much better antibacterial activities in contrast to aqueous extract which may be because of organic nature of ethanol and also for the reason of its high capacity to dissolve more organic and active antimicrobial compounds.

In another study Joshua *et al*. (2012) showed that the ethanolic extract of *Cymbopogon citratus* stem inhibited the entire tested organisms more than the leaves with varied concentrations. Whereas, *in vitro* studies of Hindumathy (2011) showed that the methanolic and aqueous extracts of *C. citrates* inhibited bacterial growth of *E. coli, P. vulgaris and S. aureus*, but their effectiveness varied with the concentration.

Leaves petroleum ether, chloroform, alcohol and aqueous extracts showed good antibacterial effects (Patra *et al.,* 2008). Mohapatra *et al.* (2011) showed that chloroform extract is very effective against *Vibrio cholerae* 811, *Shigella boydii* 81 and *Bacillus licheniformis* 10341 compared to other bacteria strains *Escherchia coli* L. row, *Shigella sonnei* 2, *Salmonella typhi* 59, *Staphylococcus aureus* 29737, *Vibrio alginolyteus*, *Salmonella typhimurium* NCTC74, *Vibrio cholera* 854 and *Staphylococcus aureus* 29737.

A study by Jani *et al.* (2012) showed that stem methanol extract of *H. auriculata* is more active on *B. subtilis* bacterial strain, while leaves and flowers methanol extracts showed good average inhibitory concentration on *Proteus vulgaris.*

The ethanolic extract of *Peristrophe bicalyculata* was more effective against *Escherchia coli* (with a largest inhibition zone = 18 ± 0.8 mm), *Bacillus cereus* and *Salmonella typhi* (Janakiraman *et al.,* 2012).

The aqueous extract (20mg/mL) of the plant is effective against *Escherchia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. A yellow-brown essential oil can

be extracted by distillation, shows *in vitro* activity against the growth of various strains of *Mycobacterium tuberculosis* (Burkill, 1985).

The leaves ethanolic macerated of *Peristrophe bicalyculata* showed greater inhibition *in vitro* against *Staphylococcus aureus*, *Klebsiella spp*, *Pseudomonas aeruginosa*, *Aspergillus Niger*, *Rhizopus stolonifer* and *Aspergillus clavatus* .

Ali *et al*., (2001) determined the antibacterial and antifungal activities of various solvent extracts of *Piper longum* L. against a wide variety of pathogenic bacteria and fungi respectively. Crude extracts of *Piper longum* L. showed mild to moderate activities against most of the tested bacteria. On the other hand, the antifungal activities exhibited by all the crude extracts were not prominent.

Petroleum ether extracts of *Piper longum* L. were found to be inactive against most of the tested organisms. Ethyl acetate extracts showed relatively better anti-microbial effect against most of the tested organisms. It has been expected that the antimicrobial screening of this plant materials will lead the scientist to continue work against pathogens causing killer diseases.

Udgire and Pathade (2014) have investigated the antimicrobial activity of ethanol and methanol extract from *Piper longum* L. rhizome against major skin pathogens *Staphylococccus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, and Proteus mirabilis*. The most promising activity of methanol extract was observed against *Klebsiella pneumonia, Proteus vulgaris, and Staphylococccus aureus* with zone of inhibition of 17mm diameter. Further bioactivity-guided fractionation of methanol extract by silica *gel* column chromatography resulted in eleven major fractions. Among these, fraction-1, 4, 5 and 6 showed higher antimicrobial activity against selected skin pathogens.

VI. SUMMARY AND CONCLUSION

In recent years the popularity of complementary medicine has increased. Over 50% of all modern drugs are natural product origin and they play an important role in drug development programmes of the pharmaceutical industry.

The present investigation elucidates the antioxidant and antimicrobial activities of *Hygrophila schulli*. Phytochemical analysis showed the presence of alkaloids, flavonoids, phenols, sterols, phenols and quinones.

A knowledge of the chemical constituents of plant is desirable for the synthesis of complex chemical substances and for discovering the actual significance of folklore medicine. Identification of secondary metabolic fingerprint by chromatography and spectroscopy tools provides useful information about qualitative, quantitative and pattern of composition of these molecules.

The UV-Visible spectrum of the methanol extract showed major and minor peaks indicating the presence of multiple components. FT-IR spectrum showed the presence of phenols, alkanes, alkenes, amines and aromatics. XRD analysis indicated the presence of sets of lattice planes and further. The observed peak broadening and noise were indicating the presence of macromolecules present in the plant extract.

GC-MS analysis showed 50 volatile organic compounds from *Hygrophila schulli*. The major compounds identified were octadecatrienoic acid, stigmasterol, hexadecanoic acid, phytol, octadecanoic acid and nonanoic acid. These information can be used to establish parameters on the development of phytotherapeutic products, helping to ensure its quality and, therefore, its safety and efficacy.

The free radical scavenging activity of *Hygrophila schulli* have showed that the efficiency of plant species differ depending on the particular assay methodology, reflecting the complexity of the mechanisms involved in total antioxidant capacity. The results of the assay are expressed in scavenging activity

of DPPH free radical expressed in percentage. The analysis showed that the radical scavenging activity of the extracts of MEHS increases with increase in concentration.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability is generally associated with the presence of reductones which breaks the free radical chain by donating a hydrogen atom. The reducing power of different concentration of *Hygrophila schulli* was found to be remarkable in this study and the absorbance of each concentration was found to rise as the concentration gradually increases.

Among many proposed strategies, a good understanding of systematic screening of traditional system of medicine offers the potential of developing potent broad spectrum antibiotics. Hence, in this present study, same efforts are continued in the progression of searching novel therapeutics against antibiotic activity. The bioactive compounds present in *Hygrophila schulli* showed a promising activity profile against *Bacillus subtilis, Staphylococcus aureus, Escherchia coli, Klebsilla sp., Salmonella typhi, Proteus sp.,* etc.

The results revealed the presence of medically important constituents in the plant studied. It is suggested that further work should be carried out to elucidate the possible mechanism of action of these extracts.

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