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Acute toxicity of the ethanol extract of aerial roots of *Rhaphidophora aurea* twined over *Lawsonia inermis*

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ABSTRACT

This study was performed to elucidate the possible toxic effect of the ethanolic extract of aerial roots of *Rhaphidophora aurea* twined over *Lawsonia inermis* on Swiss mice. Mice were orally administrated single dose of 100,250,500, 750, 1000 and 2000 mg/kg of ethanol extract and the mortality was observed for 14 days post treatment of extract. These results state that ethanol extract of *Rhaphidophora aurea* twined over *Lawsonia inermis* is toxicologically safe by oral administration.

Key words: Acute toxicity, *Rhaphidophora aurea*, LD_{50}

NTRODUCTION

Acute toxicity studies in animals is usually necessary for any pharmaceutical intended for human use. Acute toxicity is usually defined as the adverse change(s) occurring immediately or a short time following a single or short period of exposure to a substance or substances or as adverse effects occurring within a short time of administration of a single dose of a substance or multiple doses given within 24 hr. An adverse effect is "any effect that results in functional impairment or biochemical lesions that may affect the performance of the whole organism or that reduce the organ's ability to respond to an additional challenge" (1). Consequently, a chemical that enters the organism via the oral route during a restricted time and produces any adverse effect with little delay is orally and acutely toxic. However, the term acute oral toxicity is most often used in connection to lethality and LD_{50} determinations (2).

A lethal dose (LD) is an indication of the lethality of a given material of radiation. Because resistance varies from one individual to another and hence the 'lethal dose' represents a dose (usually recorded as dose per kilogram of subject body weight) at which a given percentage of subjects will die. The most commonly- used lethality indicator is the LD_{50}, a dose at which 50% of subjects will die. The OECD guidelines define the acute, subacute and chornic toxicity of the herbs and modern medicines (3).

The evaluation of the toxic action of the plant extracts is indispensable in rder to consider a treatment safe; it enables the definition of the intrinsic oxicity of the plant and the effects of acute overdose. Laboratory mice are sensitive to toxic substances occurring in plants. The administration of the extracts in increasing amounts enables the evaluation of the toxicity limits and the test should be carried out in two ways, for three doses and for both sexes, taking into account such factors as age sex, weight, species, diet and environmental conditions (4)

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An acute toxicity test can give more information about the biologic properties of a chemical compound than any other single test, and even if the incidence of lethality were never computed as a consequence of such a test, one would only have lost a small proportion of the available information (5). The purposes of acute toxicity testing are to obtain information on the biologic activity of a chemical and gain insight into its mechanism of action. Long-term studies usually start with a dose-finding exercise under acute conditions.

This plant *Rhaphidophora aurea* (*Epiperenum aureum* or *Pothos aureus*) is widely used as an indoor plant climbing from pots and hanging from baskets (6). It is very suitable as a climbing vine on trellis, poles, fences, trees (7). The toxic component of the plant has not been identified, but it is suspected that the primary irritant is calcium oxalate needles (8). So the present study is aimed to find the toxicity of ethanolic extract of *Rhaphidophora aurea* twined over *Lawsonia inermis*.

MATERIALS AND METHOD

Collection of plant materials

Aerial roots of *Raphidophora aurea* intertwined over the *Lawsonia inermis* (Mehandhi) was collected from Palakkad District and the botanical identification was carried by Botanical survey of India, Coimbatore.

Extraction

Defatted plant material 300g was extracted with ethanol and the extracts was concentrated using Equitron rotary flash evaporator and preserved in refrigerator for further use.

Phytochemical test

The extracts were tested for the presence of alkaloids, saponin, steroids, glycosides, flavonoids, terpenoid, tannins, polyphenolic compounds, anthraquinones, cyanogenic glycosides, carbohydrates, rotenone, fixed oils, fats, and volatile oils according to standard procedure(9)

Animals

Healthy young adult Swiss albino mice (25-30g), nulliparous, non-pregnant female animals were kept in group of 6 per cage. Ethical committee of KMCH College of Pharmacy, Coimbatore-48 approved the protocol for these experiments under number KMCRET/PhD03/2010-11.

Experimental groups

In order to study the possible toxic effect or changes in normal behaviour six groups (each group contain 6 mice) of mice were used in this experiment. Before commencing the experiment each animal was assigned a unique identification marking with paint like head, tail, body, head and body and no mark (Image 1).

Housing and feeding

Animals were facilitated with standard temperature (23 ± 2°C)-controlled environment (12 h: 12h (light: dark cycle)). The standard laboratory animal food pellets with water *ad libitum* feed was supplied to animals during the study period.

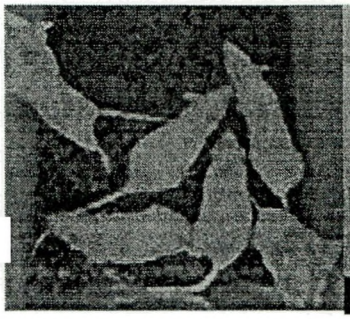

Image 1

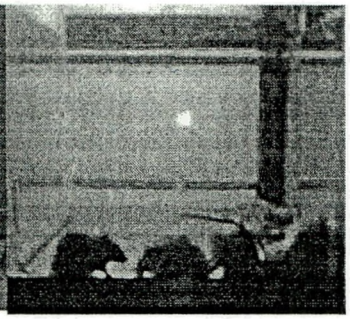

Image 2

Fixed dose procedure

The fixed-dose procedure was first proposed by the British Toxicology Society in 1984 (10). After an international validation study involving 20 reference chemicals tested in 31 laboratories from 11 different countries (11), the procedure was incorporated into the OECD guidelines (guideline 423) in 1996 (12). The results of the validation study showed a remarkable consistency between laboratories and it was concluded that the data generated could be used both for risk assessment and ranking chemicals for classification. Further evaluation of the method has proven its usefulness (13-16). The test substance is given at one of the four fixed-dose levels (100,250,500, 750, 1000 and 2000 mg/kg) to female mice. The objective is to identify a dose that produces clear signs of toxicity but no mortality. Depending on the results of the first test, either no further testing is needed or a higher or lower dose is tested: If mortality occurs, retesting at a lower dose level is necessary (except if the original dose chosen is 100 mg/kg). If no signs of toxicity occur at the initial dose, it is necessary to retest at a higher dose level. The results are thus interpreted in relation to animal survival and evident toxicity (17) and it becomes possible to assign the chemical to one of the OECD classification categories. No drug is used clinically without its clinical trials and toxicity studies.

The extract was administered in a single dose by using specially designed mice oral needle. Animals were deprived of food 3 h prior to dosing. Following the period of fasting the animals were weighed and distributed into six treated group. Group 1, 2, 3, 4, 5 and 6 were orally administered the dose of 100,250,500, 750, 1000 and 2000 mg/kg body weight of test substance. After the extract administration, food was withheld for 2 hours.

Observation parameters

Tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were direct observation parameters and some additional parameters like skin, fur, eyes, mucous membrane, respiratory, circulatory, autonomic, central nervous systems, somatomotor activity and behavior pattern were also observed.

Animals were observed (Image 2) individually after atleast once during the first 30 minutes, periodically during the first 24 hrs, with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days.

RESULTS AND DISCUSSION

Herbal medicines have received greater attention as an alternative to clinical therapy and the demand for these remedies has currently increased. Experimental screening method is imperative in order to establish the safety and efficacy of traditional and herbal products and also to set up the active components of the herbal products (18).

The phytochemical investigation of ethanolic extract of *Rhaphidophora aurea* carried out by standard procedures reveal the presence of alkaloids, flavanoids, tannins, glycosides, phenols and anthraquinone. The extracts inhibit the growth of bacteria and fungi a sign of its broad spectrum antimicrobial potential which may be used in the management of microbial infections (9). A similar species *Epiprenum pinnatum* is reported to have significant anti cancer activity (19).The importance of this plant in folk medicine as well as promoting pharmacological properties, make studies about its toxicity very important.

The oral administration of ethanolic extracts of *Rhaphidophora aurea* in dose in 100-2000 mg/kg did not induce significant changes in direct observation parameters and additional parameters. During 24 h of the experiment and after the study no death occurred in any of the groups.

The complete behavioral observations in annexure I showed that in single dose, there is no adverse effect of extract. Writhing reflux was noted in a single dose of 2000mg/kg and this reflux was absent in the previous doses.

Table I: Acute toxicity test of the ethanolic extract of *Rhaphidophora aurea* in Swiss mice

S.N	Response	No mark		Head		Body		Tail		Head & Tail		Head & Body	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Grooming	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	Touch response	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
4	Torch response	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	Pain response	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Tremors	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
7	Convulsion	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
8	Righting reflux	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
9	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	Pinna reflux	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	Corneal reflux	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
12	Writhing	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present
13	Pupils	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
14	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
15	Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
16	Skin colour	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
17	Lacrimation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
18	Hyper activity	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

In acute toxicity study there was no any mortality observed upto the maximum dose level 2000mg/kg body weight of the ethanol extract administered orally, which is the single high dose recommended by OECD guidelines-423 for testing acute toxicity.

Thus, our test suggested that ethanolic extract of *Rhaphidophora aurea* does not cause any apparent acute toxicity, since there were no significant changes in animal at all the doses of treated mice.

CONCLUSION

In the acute toxicity test a dose of 2000 mg/kg of ethanolic extract of *Rhaphidophora aurea* did not cause mortality in mice during 14-days observation. The mice did not show any signs of toxicity or change in general behaviour or other physiological activities.

The results of present study has shown that acute administration of ethanol extract of *Rhaphidophora aurea* may be safe as the LD_{50} could not be determined at the doses given. This study emphasizes the call for carrying out toxicity studies even in natural plant products and drug of indigenous medicinal system.

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A Review on *Kedrostis foetidissima(jacq.)cogn*

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ABSTRACT

The use of the medicinal herbs for curing disease has been documented in history of all civilizations. Before the onset of synthetic era, man was completely dependent on medicinal herbs for prevention and treatment of ailments. With introduction of scientific procedures the researchers, were able to understand about toxic principles present in the green flora. The scientists isolated active constituents of the medicinal herbs and after testing, some were found to be therapeutically active. *Kedrostis foetidissima (jacq.)cogn.*, (*Cucurbitaceae* family) is a traditional herb and has various activities. In this review, an overview of *Kedrostis foetidissima (jacq.)Cogn.* with respect to botany, ethno medicinal practices, traditional use, nutritional, antinutritional evaluation and antibacterial activity are given.

Key words: *Kedrostis foetidissima (jacq.)Cogn. Cucurbitaceae*; antimicrobial.

1. INTRODUCTION:

Plants have been an integral part of life in many local communities for food and medicine both[1]. Medicinal herbs are moving from fringe to mainstream use with a great number of people seeking remedies and health approaches free from seeking side effects caused by synthetic chemicals [2]. Worldwide over 80% of the people depend on medicinal plant species to meet their day today health care [3]. Medicinal plants used as sources for extracts or pure products for therapeutic use represent a rapidly expanding area of health science [4]. Higher plants, as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. It is reported that over 50% off all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in Pharmaceutical industry [5].

2. Botanical aspects:

2.1. Family Description:

The Cucurbitaceae are mostly prostrate or climbing herbaceous annuals comprising about 90 genera and 700 species that are further characterized by commonly having 5-angled stems and coiled tendrils . The leaves are alternate and usually palmately 5-lobed or divided ; stipules are absent. The flowers are actinomorphic and nearly always unisexual. The perianth has a short to prolonged epigynous zone that bears a calyx of 3-6 segments or lobes and 3-6 petals or more frequently a 3-6-lobed sympetalous corolla. The androecium is highly variable, consisting of basically 5 distinct to completely connate stamens that frequently are twisted, folded or reduced in number. The gynoecium consists of a single compound pistil of 2-5 carpels, generally with one style and as many style branches or major stigma lobes as carpels, and an inferior ovary with one locule and usually numerous ovules on 2-5 parietal placentae or 3 locules with numerous ovules on axile placentae. The fruit is a type of berry called a pepo. (Gerald Carr) [6].

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2.2. Members of the genus *Kedrostis:*

Zipcode Zoo has pages for 58 species, subspecies, varieties, forms, and cultivars in this genus:

K. abdallai · K. africana · K. angulata ·K. bainesii · K. bennettii · K. brevispinosa · K. capensis K. cinera · K. cinerea · K. cogniauxii · K. courtallensis · K. crassirostrata · K. cufodontii · K. digitata · K. dissecta · K. elongata · K. emetocathartica · K. eminens · K. engleri - K. foetidissima · K. gijef · K. gilgiana · K. glauca · K. glomeruliflora · K. gracilis ·K. grossulariaefolia · K. grossulariifolia · K. heterophylla · K. hirta · K. hirtella · K. lanuginosa K. laxa · K. ledermannii · K. leloja · K. limpompensis · K. longipedunculata ·K. macrosperma · K. malvifolia ·K. mildbraedii · K. minutiflora · K. mollis · K. nana · K. natalensis · K. obtusiloba · K. otaviensis · K. perrieri · K. psammophila · K. pseudogijef · K. punctulata · K. rautanenii · K. rigidiuscula · K. rostrata · K. schlechteri · K. sphenoloba · K. spinosa · K. triloba · K. velutina · K. zeyheri. (Cogn. Publication : Monogr. Phan. iii. 634) [6].

2.4. *Kedrostis foetidissima*- confound with other species:

Members of the genus Solena are easily recognized in the field by comparatively short petioles, with the blade more or less embracing the stem. In Southern India and Sri Lanka this character has led to incidental confusion of sterile *S. amplexicaulis* and *S.Umbellata* with *Kedrostis foetidissima (synonym K.rostrata),*all three species superficially look very much alike, because of short-petioled leaves. Furthermore, *Kedrostis foetidissima and S. amplexicaulis* both have rather similar beaked fruits. Therefore, K. *foetidissima* is included in the key to the species. The genus *Kedrostis* contains several species in Madagascar and Africa and two species in Asia. Asian *Kedrostis* can also be confounded with *Corallocarpus.* The fruits of most *Kedrostis and Solena* are indehiscent. The petioles of *Corallocarpus* and *Kedrostis (except K.foetidissima)* are comparatively long. In S India and Sri Lanka *K. foetidissima* may be confused with *Solena amplexicaulis,* both with rostrate fruit [7].

2.5. Key to the species *Kedrostis foetidissima (jacq.)Cogn:*

Kedrostis (Cucurbitaceae) occurs in Africa and Madagascar and comprises *4 (5)* species in Asia. Of these, 2 species are found in India and Sri Lanka and 2(3) species in western Malesia. *Kedrostis Medik* is an Old World genus occurring in Africa and Madagascar with c.20 species and in SE Asia with 5 species. In Asia the distribution is restricted to two separate areas:2 species(of

which *K.foetidissima* also in Africa)in S India and Sri Lanka and 3 in West Malesia. *Kedrostis bennettiii* is offensively smelling when crushed, similar as is known for the Indian *K.foetidissima(Jacq.)Cogn.*

Kedrostis foetidissima (Jacq.)cogn.(1881)634 ;(1916)140;(C.Jeffrey (1967)137,fig23,11); (Matthew(1982) plate 298:1-4 & 7-14); (1983)645.

Trichosanthes foetidissima Jacq.(1789)341;(1790) t.624.-Rhynchocarpa foetida Schrad., nom.illegit.: (C.B.Clarke (1879)627). Type: a plant sent from W.Africa, cultivated at Vienna and depicted in Jacq.1790: t.624 [8].

3. Ethno medicinal practices and traditional use :

Many of the plants that are used by *Sirumalai paliyan tribes* are mentioned in ancient literature such as Sidha, Ayurveda, Unani. The use of medicinal plant species in treating a particular ailment is also fairly common among *Paliyan tribes* living in other parts of Tamilnadu [9-11]. It was reported that, five drops of juice of the leaf of *Kedrostis foetidissima(jacq.)cogn., Cucurbitaceae,* locally named as Appakovai, given orally to treat common cold in children [12]. It was also stated that, the medicinal use of *Exacum pedunculatum Linn., Kedrostis foetidissima(jacq.)cogn., Tarenna asiatica (Linn.) Alston* and *Theriophonum fischeri M. sivadasan* are being reported for the first time in this report as there is no such record in literature [13-18].

3.1. Wild edible plants as a food source:

Utilization of wild edible plants as a food source is an integral part of the culture of indigenous people that dwell in the rain forests of Africa and South America [19-22]. who gathered and consume wild edible plants as snacks and at times of food scarcity [23-28] . Thirty-eight wild plant species were reported as sources of food by Kara and Kwego informants; among these, the leaf of *Kedrostis foetidissima(jacq.)cogn., Cucurbitaceae,* climber, was taken in boiled form [29].

3.2. *Kedrostis*- in the treatment of opportunistic infections:

Opportunistic infections are treated with multi-plant extracts of *Mangifera indica, Eucla natalensis, Carissa edulis, Psidium guajava, Pennisetum purpureum, Cymbopogon citratus , Punica granatum, Musa sp, Kedrostis foetidissima, Withania somnifera, Acacia robusta, Eucalyptus sp. Ximenia caffra, Clerodendrum mrycoides* and *Dichrostachys cinerea*[30]. The medicinal herb *Kedrostis foetidissima (jacq.)cogn.*, and *P.vogelii.* are recommended for further pharmacological test on HIV cases and for domestication to serve them from local extinction[31].

3.3. *Kedrostis*- in the treatment of Measles:

Ethnobotanical study on 71 medicinal plant species of cultivated and wild types , greatly utilized for treating a total of 41 different ailments by people of Ngai and Otwal sub countries, reveal that, roots are the most commonly harvested plant part of the medicinal plant species compared to any other part. The roots of *Kedrostis foetidissima (jacq.)cogn.* crushed, mixed in cold water is taken once a day for the treatment of Measles [32].

3.4. *Kedrostis*- in the treatment of chest pain and as a veterinary medicine:

An ethnobotanical survey on the use of medicinal plants by the Zay people of Ethiopia, revealed a total of 33 species of medicinal plants. Of the 33 medicinal plants, 10 were reportedly scarce locally, which includes *Kedrostis foetidissima (jacq.)cogn.* The whole plant of *Kedrostis foetidissima (jacq.)cogn.*, locally named as holobido(Or.) is taken orally for curing chest pain and its leaves are used as a traditional veterinary medicine in the treatment of ALOYE (a cattle disease) [33]. An ethnodiagnostic study, at Kenya reports *Kedrostis foetidissima (jacq.)cogn.*, as a rare plant with a very unpleasant smell but cattle feed on it ravenously. The leaves are crushed and fed to cattle suffering from pasture bloat and frothy bloat [34].

3.5. *Kedrostis*- in the treatment of diarrhoea and measles:

The leaf juice of *Kedrostis foetidissima*, locally named as *Appakovai*, applied externally on joints cures diarrhoea in babies of 3-4 months [35]. Medicinal use, preparation and administration modes of 299 plant species belonging to168 genera in 68 families medicinal plants of Bulamogi in Uganda shows a record , that *Kedrostis foetidissima*, wild herb, was used in treatment of diarrhoea and measles. Leaf infusion was taken in treating diarrhoea and leaf decoction was taken orally in the treatment of measles [36].

4.Nutritional and antinutritional values:

Data on proximate composition of the edible tubers, rhizome, corm, roots and stems of 23 plants consumed by tribal Valaiyans of Madurai, reveals that the tubers of *Kedrostis foetidissima* and stem of *Caralluma pauciflora* have more crude protein than the other plants. The tubers of *Kedrostis foetidissima* have higher vitamin, niacin content and more starch content. All the investigated wild edible plants appeared to have a higher level of iron content compared to Recommended dietary Allowances (RDA) of NRC/NAS(1980) for infants and adults , have low *in vitro* protein digestibility, exhibit variations in the levels of total free phenolics, tannins, hydrogen cyanide, total oxalate, amylase and trypsin [37] .

5. Antimicrobial activity:

The chloroform extract of leaf and stem of *Kedrostis foetidissima* showed significant antibacterial activity against bacteria like *Streptococcus aureus, Escherichia coli, Pseudomonus aeruginosa, Klebsiella pneumonia, Serratia marcescens.* The maximum zone of inhibition was found in stem against *Pseudomonus aeruginosa(12.6mm)* and the minimum zone of inhibition was found in leaf extract against *Escherichia coli(3.5mm).* Since the plants used in this study have proved to possess antimicrobial properties, and are locally available , they may become alternative sources of antimicrobial drugs that will complement existing antibiotics and are provide novel or lead compounds that may be employed in controlling infection [38].

Water extracts of the aerial parts of *Kedrostis foetidissima* a succulent plant, belonging to the Cucurbitacae family have been used for centuries by Kenyans as an effective remedy for complications arising from the measles virus infection in children. The study shows that this extract is encouragingly active against a number of bacterial species: *Klebsiella pneumonia, Escherichia coli, Streptococcus aureus, Pseudomonus aeruginosa,Shigela flexnieri,Vibrio Comma, Salmonella typhi, Streptococcus pneumonia* and *Enterobacter aerogines.* The extract is also active against the measles viru *Leishmania denovani,* the *visceral Leishmania Parasite,* as well as *Trypano soma brucei* [39].

6. Bio activity and domestication:

Bio activity test in millimeter for the ethanol extracts of the *Kedrostis foetidissima* against the test microbes, *Escherichia coli; Salmonella typhi; Staphylococcus aureus ; Pseudomonus aeruginosa; Streptococcus faecalis; Klebsiela pneumonium, Candida albicans* showed no significant difference between the wild and the domesticated plant extracts despite the difference in soil characteristics [32].

A grove in Kanchipuram district protects rare species like Amorphophallus Sylvaticus, *Kedrostis foetidissima* and also a huge 200 year old banyan tree which provides a shady atmosphere [40].

7.Chemical constituents:

Preliminary studies on this plant in our laboratory reveal the presence of

terpenoids, sterols , amino acids . Several of non- protein amino acids were isolated first from members of *Cucurbitaceae,* [41] and the survey was undertaken to gain information concerning the generality of their occurrence within the members of the family .Normally seeds have been examined in preference to vegetative organs for their composition is likely to be affected by variation on nutritional or environmental factors during plant growth. The results obtained were examined in relation to the classification of the family recently compiled by Jeffery
(1961 &1964) [42, 43].

Each seed species was ground and extracted with 75%(v/v) ethanol and then the amino acid fraction was separated from the other aqueous-ethanol soluble constituents by absorption upon a cation- exchange resin. Individual amino acids were identified after separation on 2- Dimensional chromatograms prepared from each extract. *Kedrostis foetidissima,* shows mild chromatographic spot for the presence of amino acid, Citrulline (I) and traces of *m-Carboxyphenylalanine* . It also shows spots for the presence of traces of unknown Ninhydrin positive compounds [44].

8. CONCLUSION:

Among the 58 species of the genus *Kedrostis*, *Kedrostis foetidissima* distributed all over the world and have immense medicinal values traditionally. Various crude extracts of *Kedrostis* were found to have anti microbial properties and also recommended for further pharmacological test on HIV cases. The reports on isolation studies of compounds from this plant are sparse. This herbal plant, which is rare in existence can be easily domesticated and is expected to have several medicinal properties. This review is aimed at studying the potential of *Kedrostis foetidissima* and henceforth isolation of compounds from its stem and leaves.

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