PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *KEDROSTIS FOETIDISSIMA* (JACQ.) COGN.

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ABSTRACT

Hexane, petroleum ether, chloroform, acetone and methanol extracts of leaf, stem and tuber of *Kedrostis foetidissima* were tested for qualitative and quantitative phytochemical analysis. The results of *K. foetidissima* leaf extracts revealed the presence of flavonoids and steroids in all extracts studied. Flavonoids, tannins, triterpenoids, phenols, steroids, glycosides and cardiac glycosides are present in the chloroform, methanol and acetone extracts while saponin were detected only in methanol and acetone extract. The various extracts were evaluated for antibacterial activities. Antibacterial activities of the extracts against *Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumoniae, Serratia marcescens* and *Pseudomonas aeruginosa* were investigated by using disc diffusion method. It was observed that, chloroform, acetone and methanol extracts of leaf, stem and tuber of *Kedrostis foetidissima* showed activity against the entire tested gram positive and gram negative bacterial strains. Inhibitory effect of 100% concentration of petroleum ether and hexane extract was observed against *Serratia marcesense* (7.05; 4.00mm), *Staphylococcus aureus* (5.67; 6.83mm), *Escherichia coli* (4.93; 4.40mm) and *Pseudomonas aeruginosa* (2.15; 3.50mm). Hence, this plant can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals that address unmet therapeutic use.

Key words: *Kedrostis foetidissima,* antibacterial activity, gram positive bacteria.

INTRODUCTION

Medicinal plants are the nature's gift to human beings to make disease free healthy life. Several millions of Indian households have been using through the ages nearly 8,000 species of medicinal plants for their health care needs. Over one and a half million traditional healers use a wide range of medicinal plants for treating ailments of both humans and livestock across the length and breadth of the country.

In India, different parts of several medicinal plants or their extracts are used for the treatment of various diseases. Several antibiotics used for the treatment of human infections, which have limited antimicrobial spectrum. They could develop drug resistance in pathogens and lead to serious ill effect. Hence plant derived antimicrobial properties have received considerable attention in recent years. Several plants have been indicated in folk and other traditional system of medicine as aseptic agents. More than a hundred species of therapeutically important higher plants are listed and described in ancient Indian treatise to have the antimicrobial activity. Efforts are thus directed to identify the plant products which have broad spectrum of antimicrobial property with no ill effects (Agrawal, 1986).

Considering all these in mind, the present study is concentrated on the medicinal plants *Kedrostis foetidissima* (Jacq.) Cogn. (Cucurbitaceae) which is very effective in the treatment of asthma, chest pain and urinary tract infection (Giday, 2001), diarrhoea, HIV (Otieno *et al.,* 2008), small pox, skin diseases (Tabuti *et al.,* 2003), snake bite (Dymock, 1891) and livestock problems (Ole Miaron, 2003). With this background, the present study was carried out to evaluate the antibacterial potential of different solvent extracts of leaf, stem and tuber of *Kedrostis foetidissima*

MATERIALS AND METHODS

PLANT MATERIALS

Different parts of leaf, stem and tuber of *Kedrostis foetidissima* were collected during Nov 2009-Feb 2010 from Maruthamalai Hills, Coimbatore, Tamilnadu, India. The collected plant materials were identified and their authenticity was confirmed by Mathew (1981) and Gambel (1986) respectively.

The voucher specimens were deposited in the Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India.

EXTRACTION OF PLANT MATERIAL

Various organic solvents were used for the extraction of bioactive compounds. The leaf stem and tuber powders (10g) of *Kedrostis foetidissima* were first extracted with petroleum ether for defatting in a Soxhlet apparatus. The defatted powdered sample of *Kedrostis foetidissima* were dried and successfully extracted with hexane, petroleum ether, chloroform, acetone and methanol in a Soxhlet apparatus. The extracts obtained were completely evaporated by using vacuum rotary evaporator. The concentrated extracts were used for antibacterial activity.

QUALITATITVE AND QUANTITATIVE ANALYSIS

The concentrated extracts were subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures (Harborne, 1984; Trease and Evans, 1989; Sofowora 1993). The flavonoid content was determined according to Jia *et al*, (1999). The steroid content was estimated by the method of Harborne (1984). The total phenols and tannin contents were estimated by the methods of Sadasivam and Manickam (2005).

TESTED MICROORGANISMS

Antimicrobial activity of crude extracts was tested against gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Serratia marcescens* and gram-positive *Staphylococcus aureus.* All the microbial cultures were procured from the Microbiology Laboratory, K.G Hospital, Coimbatore-641018. The stock cultures of bacteria were maintained on nutrient agar slants.

ANTIBACTERIAL ASSAY

Antibacterial activity was demonstrated using a modification of the method originally described by (Bauer *et al.,* 1966) which is widely used for the antibacterial susceptibility testing (Barry and Thornsberry, 1985). A loopful bacteria was taken from the stock culture and dissolved in 0.1ml of saline. All the tests were done by placing the disc (6mm diameter) impregnated with (20µl) various crude solvent extracts on the Mueller Hinton Agar surface previously inoculated with 10ml of MHA liquid medium with Gram positive and Gram negative bacteria. Respective solvents without plant extracts served as negative control. Plates were incubated at 37^{o}C for 24 hours. After the incubation period, the diameter of the inhibition zone around the plant extracts saturated discs were measured and also compared with the diameter of inhibition zone of respective solvents.

STATISTICAL ANALYSIS

Statistical analysis was performed using statistical software package WINSAT 2007 in Microsoft Excel. The data were presented as Means ± S.E. Statistical analysis was performed using one way ANOVA, DMRT test was used for calculating for 5 % level of significance.

RESULTS AND DISCUSSION

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical analysis of different solvents of various parts of *K. foetidissima* is depicted in Table 1. The results of *K. foetidissima* leaf extracts revealed that the presence of flavonoids and steroids in all extracts studied. Flavonoids, tannins, triterpenoids, phenols, steroids, glycosides and cardiac glycosides are present in the chloroform, methanol and acetone extracts while saponin were detected only in methanol and acetone extract. Petroleum ether and hexane extract contains alkaloids, flavonoids, tannins, triterpenoids (+) and steroids (++) with very lesser degree of precipitation while other phytochemical compounds like phenols, phlobatannins, anthraquinones, glycosides, cardiac glycosides and saponins are absent (Table 1). Phenols, tannins, triterpenoids, flavonoids and steroids showed moderate degree of precipitation (++) in the chloroform extract whereas alkaloids show higher degree of precipitation (+++) and the negative results were observed for phlobatannins and anthraquinones. The higher degree of precipitation (+++) of alkaloids, flavonoids, phenols, tannins, triterpenoids, glycosides, cardiac glycosides, steroids and saponins were observed in methanol extract.

In the acetone extract, flavonoids, phenols, tannin, triterpenoids and steroids showed moderate amount of precipitation (++). Glycosides, cardiac glycosides and saponin showed very lesser degree of precipitation (+). All the extracts showed negative results for phlobatannins and anthraquinones.

Table 1: Qualitative phytochemical analysis of *Kedrostis foetidissima*

Tests	**Leaf**					**Stem**					**Tuber**				
	Pet	**Hex**	**Chlf**	**Acet**	**Met**	**Pet**	**Hex**	**Chlf**	**Acet**	**Met**	**Pet**	**Hex**	**Chlf**	**Acet**	**Met**
Alkaloids:															
(i)Dragendroff's	+	+	+++	-	+++	+	+	++	-	++	+	+	++	-	++
(ii)Wagner's	+	+	+++	-	+++	+	+	++	-	++	+	+	++	-	++
(iii)Meyer's	+	+	+++	-	+++	+	+	++	-	++	+	+	++	-	++
Flavonoids:															
(i)FeCl3	+	+	++	++	+++	+	+	++	++	+++	+	+	++	++	++
(ii)Lead acetate	+	+	++	++	+++	+	+	++	++	+++	+	+	++	++	++
(iii)NaOH	+	+	++	++	+++	+	+	++	++	+++	+	+	++	++	++
(iv)Shinoda	+	+	++	++	+++	+	+	++	++	+++	+	+	++	++	++
Phenols:															
(i) FeCl3	-	-	++	++	+++	-	-	++	+	++	-	-	++	++	++
(ii)Lead acetate	-	-	++	++	+++	-	-	++	+	++	-	-	++	++	++
Tannins	+	+	++	++	+++	-	-	++	++	++	-	-	++	++	++
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Triterpenoids:															
(i)Liberman's	+	+	++	++	+++	+	+	-	-	+++	+	+	-	-	++
(ii)Salkowski	+	+	++	++	+++	+	+	-	-	+++	+	+	-	-	++
Glycosides	-	-	-	+	+++	-	-	+	+	+	-	-	+	+	++
Cardiac glycoside															
(i)Liberman's	-	-	-	+	+++	-	-	+	+	+	-	-	+	+	++
(ii)Salkowski	-	-	-	+	+++	-	-	+	+	+	-	-	+	+	++
(iii)Keller-Killani	-	-	-	+	+++	-	-	+	+	+	-	-	+	+	++
Steroids	++	++	++	++	+++	++	++	++	++	+++	+	+	++	++	++
Saponins	-	-	-	+	+++	-	-	-	-	++	-	-	-	+	++

Note: + === Less precipitation, ++ == Moderate precipitation, +++ == Higher precipitation, - == Negative test

Table 2: Quantitative phytochemical analysis of *Kedrostis foetidissima*

Samples tested	Flavonoids(mg/g)	Steroids(mg/g)	Tannins(mg/g)	Phenols(mg/g)
Leaf				
Petroleum ether	0.22^{e}±0.46	0.28^{c}±0.38	0.17^{d}±0.24	0.19^{e}±0.20
Hexane	0.32^{e}±0.27	0.53^{b}±0.44	0.17^{d}±0.24	0.28^{e}±0.09
Chloroform	1.83^{b}±0.80	1.00^{a}±0.29	1.12^{b}±0.29	1.46^{c}±0.06
Acetone	1.04^{c}±0.49	0.39^{c}±0.44	0.62^{c}±0.40	1.06^{c}±0.09
Methanol	2.56^{a}±0.50	0.69^{b}±0.26	1.68^{a}±0.35	2.28^{a}±0.49
Stem				
Petroleum ether	0.28^{e}±0.21	0.23^{c}±0.45	0.15^{d}±0.35	0.28^{e}±0.95
Hexane	0.22^{e}±0.26	0.32^{c}±0.38	0.17^{d}±0.34	0.26^{e}±0.35
Chloroform	1.22^{c}±0.46	0.69^{b}±0.35	0.54^{c}±0.64	0.68^{d}±0.21
Acetone	0.70^{d}±0.35	0.54^{b}±0.35	0.57^{c}±0.84	0.52^{d}±0.20
Methanol	1.55^{b}±0.61	0.59^{b}±0.21	0.90^{c}±0.45	0.95^{d}±0.69
Tuber				
Petroleum ether	0.25^{e}±0.49	0.37^{c}±0.52	0.11^{d}±0.15	0.18^{e}±0.26
Hexane	0.33^{e}±0.45	0.45^{c}±0.46	0.18^{d}±0.23	0.20^{e}±0.20
Chloroform	1.08^{c}±0.55	0.97^{b}±0.15	0.65^{c}±0.26	1.16^{c}±0.60
Acetone	0.87^{d}±3.82	0.52^{b}±0.55	0.58^{c}±0.29	0.92^{d}±0.59
Methanol	1.79^{b}±0.81	0.54^{b}±0.46	0.98^{c}±0.30	1.51^{b}±0.26

Values are expressed as Mean±Standard Error of 3 replicates. Means followed by a common letter aren't significantly different at the 5% level by DMRT

The petroleum ether and hexane extract of *K. foetidissima* stem showed very lesser amount (+) for alkaloids, flavonoids, triterpenoids and steroids while negative results obtained for tannin, phenols, phlobatannins, anthraquinones, glycosides, cardiac glycosides and saponins. The chloroform, methanol and acetone extracts showed the presence of flavonoids, phenols, tannins, glycosides, cardiac glycosides and steroids. The phytochemical compounds present in the chloroform extract showed moderate degree of precipitation (++) except cardiac glycosides and glycosides (+) whereas in acetone extract phenols, glycosides and cardiac glycosides showed very lesser degree of precipitation (+) while flavonoids, tannins and steroids showed moderate degree of precipitation (++). In the methanol extract, higher degree of precipitation (+++) was noted for flavonoids, triterpenoids and steroids. Alkaloids, phenols, tannins and saponin showed moderate degree of precipitation (++). Similarly *to K. foetidissima* leaf extracts, stem extracts also showed negative results for phlobatannins and anthraquinones in all extracts (Table 1).

K. foetidissima tuber chloroform extract revealed moderate degree of precipitation (++) for alkaloids, flavonoids, tannins, phenols, steroids, glycosides and cardio glycosides (Table 1). Alkaloids, flavonoids, phenols, tannins, steroids, cardiac glycosides and glycosides were present in both chloroform and methanol extract while saponins were detected only in methanol and acetone extract. Petroleum ether and hexane extract showed positive results only for alkaloids, flavonoids, triterpenoids and steroids. Other phytochemical compounds are absent. Acetone extract show moderate degree of precipitation (++) for flavonoids, phenols, tannin, steroids followed by and saponins, cardiac glycosides and glycosides (+).

Table 3: Antibacterial activity of *Kedrostis foetidissima*

Sample tested	Diameter zone of inhibition in mm														
	Staphylococcus aureus					*Escherichia coli*					*Klebsiella pneumoniae*				
Leaf	**C**	**25%**	**50%**	**75%**	**100%**	**C**	**25%**	**50%**	**75%**	**100%**	**C**	**25%**	**50%**	**75%**	**100%**
Petroleum ether	0.00	2.83^{h}	3.53^{i}	4.07^{i}	5.67^{l}	0.00	2.27^{h}	3.00^{f}	3.57^{g}	4.93^{h}	0.00	0.00	0.00	0.00	0.00
Hexane	0.00	3.17^{g}	4.67^{g}	5.83^{g}	6.83^{i}	0.00	2.80^{g}	3.17^{f}	3.33^{h}	4.40^{i}	0.00	0.00	0.00	0.00	0.00
Chloroform	0.00	19.20^{a}	20.75^{b}	21.77^{b}	22.13^{b}	0.00	6.83^{a}	5.00^{d}	6.53^{d}	9.53^{b}	0.00	3.00^{e}	4.87^{f}	5.33^{h}	7.43^{f}
Acetone	0.00	12.30^{c}	15.43^{c}	16.51^{c}	17.87^{c}	0.00	4.00^{e}	6.33^{c}	7.67^{c}	9.00^{c}	0.00	5.43^{c}	6.07^{d}	7.67^{d}	8.17^{e}
Methanol	0.00	18.78^{b}	21.31^{a}	23.04^{a}	24.78^{a}	0.00	4.93^{d}	6.80^{b}	8.07^{b}	11.23^{a}	0.00	4.93^{d}	6.77^{c}	7.23^{e}	10.43^{a}
Stem															
Petroleum ether	0.00	0.00	1.17^{m}	2.17^{l}	3.17^{m}	0.00	1.00^{j}	2.20^{h}	3.10^{h}	3.87^{j}	0.00	0.00	0.00	0.00	0.00
Hexane	0.00	0.00	3.00^{j}	4.20^{i}	5.73^{j}	0.00	1.50^{i}	2.93^{g}	3.66^{g}	4.83^{h}	0.00	0.00	0.00	0.00	0.00
Chloroform	0.00	6.17^{e}	7.50^{e}	9.00^{e}	11.00^{e}	0.00	4.33^{e}	7.13^{a}	8.80^{a}	9.00^{c}	0.00	3.00^{e}	4.50^{f}	5.87^{g}	6.07^{g}
Acetone	0.00	2.00^{i}	3.33^{j}	5.13^{h}	7.67^{h}	0.00	3.80^{f}	5.20^{d}	6.67^{d}	7.33^{f}	0.00	2.50^{f}	5.20^{e}	6.97^{f}	7.20^{f}
Methanol	0.00	7.17^{d}	8.83^{d}	11.00^{d}	12.03^{d}	0.00	5.66^{c}	7.00^{a}	7.66^{c}	9.07^{c}	0.00	6.93^{a}	8.53^{a}	9.13^{a}	9.87^{b}
Tuber															
Petroleum ether	0.00	1.00^{k}	2.50^{k}	3.10^{k}	4.20^{l}	0.00	0.00	1.53^{i}	2.37^{i}	3.90^{j}	0.00	0.00	0.00	0.00	0.00
Hexane	0.00	1.77^{j}	2.00^{l}	3.67^{j}	5.00^{k}	0.00	0.00	0.00	4.27^{f}	5.00^{g}	0.00	0.00	0.00	0.00	0.00
Chloroform	0.00	5.69^{d}	6.97^{f}	8.67^{f}	10.00^{g}	0.00	5.67^{c}	6.17^{c}	7.50^{c}	8.43^{d}	0.00	4.70^{d}	5.10^{e}	8.00^{c}	9.07^{c}
Acetone	0.00	2.08^{i}	4.00^{h}	5.00^{h}	7.67^{h}	0.00	0.00	4.97^{e}	5.93^{e}	7.67^{e}	0.00	0.00	6.20^{d}	7.60^{d}	8.97^{d}
Methanol	0.00	5.82^{d}	6.87^{f}	9.06^{e}	10.97^{f}	0.00	6.00^{b}	7.00^{a}	8.47^{b}	9.27^{c}	0.00	5.80^{b}	8.07^{b}	8.83^{b}	9.97^{b}

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Methanol leaf extract of *K. foetidissima* had high content of flavonoids, tannins and phenols (2.56±0.50; 1.68±0.35 and 2.28±0.49 mg/g) while steroid was noted in chloroform extract (1.00±0.29 mg/g). Similarly in stem and tuber extract of methanol showed maximum content of flavonoids (1.55±0.61; and 1.79±0.81 mg/g), tannins (0.90±0.45 and 0.98±0.30 mg/g) and phenols (0.95±0.69 and 1.51±0.26 mg/g) whereas steroid was inferred in chloroform extract (0.69±0.35 and 0.97±0.15 mg/g) (Table 2).

The results of phytochemical analysis of the *K. foetidissima* were investigated and are summarized in Tables 1 and 2. The results show that the plants are rich in alkaloids, flavonoids, triterpenoids and steroids. The presence of these bases in the investigated plants account for their usefulness as medicinal plants. The degree of precipitation of secondary metabolites varies in solvents. This may be due to various degrees of solubility of different solvents for different phytoconstituents.

Alkaloids are heterogeneous group compounds which contain one or more nitrogen atom in acyclic system. These are widely used in medicinal purposes which have positive and negative effects even to human beings. Most of the plants have alkaloids in different organs with different chemical configurations (Harborne, 1984). Alkaloids are reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pains, developed resistance against diseases and endurance against stress (Gupta, 1994). They also have a protective role in animals (Edeoga and Eriata, 2001). High degree precipitation of alkaloids found in the methanol and chloroform extracts of *K. foetidissima* leaves. The present result coincides with the view of Jain *et al.,* (2004) who found high degree of alkaloid precipitation in methanol and chloroform extracts of *Cocculus hirsutus*. Many researchers reported that the presence of alkaloids in the plants cure asthma (Mary, 2009), snake bite (Li *et al.,* 1999) and skin diseases (Xu and Lee, 2001). The presence of alkaloid may be the reason why the infusion of leaves of *K. foetidissima* are given orally in village areas to cure asthma, skin diseases and snake bite. Flavonoids are 15 carbon compounds generally distributed throughout the plant kingdom (Harborne, 1988). Flavonoids have been refereed to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergies, virus and carcinogens. They show antiallergic, anti-inflammatory, antimicrobial and anticancer activity (Aiyelaagbe and Osamudiamen, 2009). Flavonoids are found in methanol, chloroform and acetone extracts of different parts of *K. foetidissima* and shown different degree of precipitation. Highest content of total flavonoids was quantified in the methanol extracts of leaves of *K. foetidissima* followed by its tuber and stem. This results can be correlated with the result of Siciliano *et al.,* (2004) who detected and quantified eight flavonoids, three C-glycosyl and five O-glycosyl flavones in roots, leaves, stems and fruits of *Sechium edule* and reported highest amount of total flavonoids in the leaves, followed by roots and finally by stems.

Table 4: Antibacterial activity of *Kedrostis foetidissima*

Sample tested	**Diameter zone of inhibition in mm**									
	Serratia marcesense					***Pseudomonas aeruginosa***				
Leaf	**C**	**25%**	**50%**	**75%**	**100%**	**C**	**25%**	**50%**	**75%**	**100%**
Petroleum ether	3.00^{d}	5.09^{i}	6.00^{g}	6.25^{h}	7.05^{i}	0.00	1.20^{d}	1.45^{g}	2.00^{g}	2.15^{f}
Hexane	1.00^{f}	1.02^{j}	2.15^{h}	3.67^{i}	4.00^{j}	0.00	1.00^{d}	2.10^{f}	3.07^{f}	3.50^{e}
Chloroform	3.67^{c}	18.00^{c}	21.00^{b}	23.00^{b}	24.67^{b}	0.00	3.63^{c}	5.33^{d}	7.00^{b}	9.00^{a}
Acetone	3.47^{d}	19.93^{b}	21.10^{b}	22.17^{c}	23.36^{c}	0.00	3.60^{c}	5.87^{c}	7.83^{a}	9.40^{a}
Methanol	4.00^{b}	21.30^{a}	23.00^{a}	26.00^{a}	28.60^{a}	0.00	5.13^{b}	6.13^{b}	7.33^{b}	9.38^{a}
Stem										
Petroleum ether	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hexane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chloroform	4.67^{a}	6.00^{g}	8.00^{f}	10.00^{f}	12.67^{e}	0.00	0.00	0.00	5.20^{d}	7.43^{c}
Acetone	3.83^{c}	7.67^{f}	9.33^{d}	11.00^{e}	12.33^{f}	0.00	0.00	0.00	4.67^{e}	6.07^{d}
Methanol	4.27^{b}	8.00^{e}	8.67^{e}	11.00^{e}	12.67^{e}	0.00	0.00	4.00^{e}	6.42^{c}	8.10^{b}
Tuber										
Petroleum ether	2.00^{e}	1.10^{j}	1.58^{l}	3.00^{j}	1.00^{l}	0.00	0.00	0.00	0.00	0.00
Hexane	2.00^{e}	0.80^{k}	1.04^{j}	1.50^{k}	1.85^{k}	0.00	0.00	0.00	0.00	0.00
Chloroform	3.33^{d}	7.67^{f}	8.67^{e}	9.00^{g}	10.67^{g}	0.00	0.00	0.00	0.00	3.53^{e}
Acetone	3.00^{d}	5.33^{h}	6.00^{g}	9.33^{g}	10.00^{h}	0.00	5.33^{a}	4.00^{e}	5.40^{d}	6.07^{d}
Methanol	3.57^{c}	10.60^{d}	11.43^{c}	12.23^{d}	13.60^{d}	0.00	0.00	7.00^{a}	6.06^{c}	8.33^{b}

Values are expressed as Mean of 3 replicates. Means followed by a common letter aren't significantly different at the 5% level by DMRT.

Phenols are reported antitumour agents and to exhibit antiviral and antimicrobial activities (Robbins, 1980), hypotensive effects (Matsubara *et al.,* 1985) and antioxidant properties (Robak and Gryglewski, 1988). Methanol extracts of leaf of *K. foetidissima* leaf showed highest phenolic content. The presence of the phenolic compounds in these studied samples proved that they had antimicrobial and antifungal effect. High degree precipitation of phenols was observed in methanol leaf extracts of *K. foetidissima*. Similar reports were reported in methanol leaf extracts of *oxalis corniculata* (Raghavendra *et al.,* 2006). High content of tannin was observed in the leaf of *K. foetidissima*. This may be the reason why most of the people are used these plants for the treatment of insect bites.

Cardiac glycosides were completely absent in hexane leaf extract of *K. foetidissima* while it was reported in methanol extract. The present report was contrary to the findings of Aiyelaagbe and Osamudiamen (2009) who observed cardiac glycosides in leaves extract of hexane and methanol of *Mangifera indica*.

The adequate report of steroids in various studies indicated their importance and interest in pharmacy due to their relationship with the compounds such as sex hormones, especially in the development of female contraceptive pill. This may be the reason why the infusion of the leaves of *K. foetidissima* are applied to the utter of cows at the time of milking, where the udder sends a message to the cow's brain to start utting down signal to squeeze the milk out of the alveoli cells. They are also given to expectant mothers and breast feeding mothers to ensure their hormonal balance, since steroidal structures could serve as potent starting material in the synthesis of these hormones. These leaves are used to prepare food for nursing mothers as medicine in some villages of Tamilnadu. High degree precipitation of steroids was found to be present in methanol leaf extract of *K. foetidissima*. The present works are in agreement with Raghavendra *et al.,* (2006) who reported the presence of steroids in methanol leaf extract of *Oxalis corniculata*. Presence of steroids was noted in the hexane leaf extract of *K. foetidissima.* The present investigations are corroborated with Ajaiyeoba (2002) and Aiyelaagbe and Osamudiamen (2009) who observed the presence of steroids in the leaves of *Parkia bicolor*, *P. biglobosa* and *Mangifera indica* respectively.

Saponins are terpene glycosides. Saponin is useful in medicine and pharmaceutical industry due to its foaming ability that produces frothy effect. Saponin is also used in the manufacture of shampoos, insecticides and various drug preparations and in synthesis of steroid hormones (Okwu, 2003). Generally saponins are toxic, but Price *et al.,* (1987) showed that consumption of saponins by human beings may be beneficial in reducing heart disease (by binding of saponins with plasma membrane and cholesterol). According to Shahidul Alam *et al.,* (2000) the presence of steroidal saponins could develop resistance to viral diseases. Finar (1989) reported that, saponins had expectorant action which is very useful in the management of upper respiratory tract inflammation. So these plants may be used to treat various ailments.

In *K. foetidissima* leaves, high degree precipitation of saponins was found in methanol extract, while it was found to be absent in chloroform extract. The present work was harmony with Ramkumar *et al.,* (2007) and Sunilson *et al.,* (2009) *Gymnema montanum* and *Codium decorticatum* leaves respectively. Methanol stem extract of *K. foetidissima* and *C. epigaeus* showed the presence of saponin, while it was absent in chloroform extract. The above result was coincidence with the report of Moorthy *et al.,* (2007) who observed saponins in methanol stem extract of *Mallotus philippinensis* and absent in chloroform extract. High degree precipitation of saponins was observed in methanol leaves extract of *K. foetidissima* and low degree precipitation in stems. Similar findings were obtained by Zabri *et al.,* (2008) in *Secamone afzelii*. The present investigation was contradictory to Nataraj *et al.,* (2009) who noted the absence of saponins in both methanol and acetone extracts of *Amorphophallus paeoniifolius*.

Methanol leaf and stem extract of *K. foetidissima* showed the presence of triterpenoids. The present works are in agreement with Akinmoladun *et al.,* (2007a,b) who identified the presence of triterpenoids in the leaves and stems of *Chromolaena odorata* and *Alstonia boonei* respectively. Mahato and Sen (1997) reported that terpenoids had wound healing properties. Dymock (1891), Kottaimuthu (2008) and Swarnkar and Katewa (2008) who reported that *K. foetidissima* were very effective in the treatment of asthma, snake bite and diarrhoea respectively.

To site this, researchers reported that the presence of terpenoids cure bronchial asthma (Vasconcelosa *et al.,* 2008), diarrhoea and bacillary dysentery (Jarukamjorn and Nemoto, 2008).

Researchers also reported the presence of terpenoids in Cucurbitaceae members (Chen *et al.,* 2009 and Lewinsohn *et al.,* 2008). The presence of triterpenoids in the studied species may cure diarrhoea and asthma.

ANTIMICROBIAL ACTIVITY

Chloroform, acetone and methanol extracts obtained from *K. foetidissima* leaf showed mild to moderate activity against most of the tested bacteria (Table 3 and 4). Inhibitory effect of 100% concentration of petroleum ether and hexane extract was observed against *Serratia marcesense* (7.05; 4.00mm), *Staphylococcus aureus* (5.67; 6.83mm), *Escherichia coli* (4.93; 4.40mm) and *Pseudomonas aeruginosa* (2.15; 3.50mm) whereas no inhibition zone was noted against *Klebsiella pneumoniae*. The results were compared with respective solvent which acted as a control. The maximum zone of growth inhibition was exhibited by the methanol extract on gram negative *S. marcesense* and gram positive *S. aureus* (28.60, 24.78mm), followed by chloroform (24.67; 22.13mm) and acetone extract (23.36; 17.87mm) when administered at 100% concentration. The chloroform, acetone and methanol extracts revealed equal inhibitory activity against *P. aeruginosa* (>9.00mm) at 100% concentration while chloroform and acetone extracts showed significantly equal activity against *E. coli* (>9.00mm) at same concentration. The chloroform and acetone extract exhibited significantly moderate activity against all test isolates. When compared all the five extracts, methanol extract showed broad spectrum of activity against all tested organisms.

The three extracts (chloroform, acetone and methanol) from *K. foetidissima* stem showed mild to moderate activity against most of the bacteria tested while 100% concentration of petroleum ether and hexane extract was found to be active against only gram positive *S. aureus* (3.17; 5.73mm) and gram negative *E. coli* (3.87; 4.83mm). The chloroform, acetone and methanol extracts revealed equal inhibitory activity against *S. marcesense* at 100% concentration similarly it showed significant activity against *E. coli*, *P. aeruginosa* and *K. pneumoniae*. On the other hand, the 100% concentration of methanol extract showed maximum zone of inhibition against *S. aureus* (12.03mm). *S. marcesense*, *P. aeruginosa* and *K. pneumoniae* showed high degree of resistance to the petroleum ether and hexane extract. With regard to antibacterial activity of different extracts of tuber of *K. foetidissima*, all the crude extracts appeared to have mild to moderate activity against most of the bacterial strains. But petroleum ether and hexane extract did not show any activity against *P. aeruginosa* and *K. pneumoniae*. Results also indicated that all the three extracts (chloroform, acetone and methanol) were found to be strongly active against gram positive *S. aureus* (10.00; 7.67 and 10.97 mm), gram negative strains *S. marcesense* (10.67; 10.00 and 13.60 mm), *K. pneumoniae* (9.07; 8.97 and 9.97 mm) and *E. coli* (8.43; 7.67 and 9.27 mm) at 100% concentration. The minimum zone of inhibition was observed against *P. aeruginosa* (3.53mm) when administered with 100% concentration of chloroform extract. The petroleum ether and hexane extract showed significantly less activity against *E. coli*, *S. aureus* and *S. marcesense*.

The results of the present study supported the therapeutical potency of *K. foetidissima*. Methanol, chloroform and acetone leaf, stem and tuber extracts of *K. foetidissima*, inhibited the growth of gram positive and gram negative bacterial strains. Similar observations were reported by Parekh *et al.,* (2005), Vaghasiya and Chanda (2007) and Doughari *et al.,* (2009). In the present investigation, the most susceptible bacterium was *S. aureus* and *P. aeruginosa*. Present results were agreed with observations in which methanolic leaf extracts of *Coccinia grandis* showed inhibitory effect on *S. aureus*, *P. aeruginosa*, *E. coli* and *K. pneumonia* (Dewanjee *et al.,* 2007; Farrukh *et al.,* 2008). In this study, fluctuating trends of inhibition zone was found to be appear in certain extracts used media contained against some pathogens (Tables 3 and 4). Similar fluctuation trends of inhibition zone was reported by Kunjal Bhatt *et al.,* (2003) and Uma and Sasikumar (2005)**.** This may be due to the fact that higher concentrations, rate of diffusion might have been varied and hence, it might not have been available to react with the microorganisms. Parekh and Chanda (2007) reported that methanolic extracts of *Lagenaria vulgaris*, *Momordica charantia* and *Mukia maderaspatana* showed inhibitory effect against *K. pneumoniae*, while no zone observed against *E. coli*. Similarly, fruits, leaves, stems and roots of *Citrullus colocynthis* showed no response against *E. coli* and *P. aeruginosa* (Memon *et al.,* 2003).

But in the present study, all parts showed inhibitory activity against *E. coli*, *P. aeruginosa* and *K. pneumoniae*. The extracts of *K. foetidissima* clearly reveal the antimicrobial nature and it is an evident that the findings of the present study can provide a basic concept for synthesizing a new drug. It will also help to isolate new antibiotic substances that control the infectious disease causing microbial pathogens.

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