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PHYTOCHEMICAL EVALUATION AND ANTIMICROBIAL ACTIVITY OF *VITEX NEGUNDO* (L.) LEAVES EXTRACTS

S. Anbuselvan*[1] and V. Muralikrishnan[1]

*[1]Department of Microbiology, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

ABSTRACT

Medicinal plants are the wealthy source of antibacterial agents and curatives. *Vitex negundo* Linn are commonly practiced medicinal plants in the villages of Tamilnadu (India). Plants grown in this region are not systematically tested for their biological activities in general and antimicrobial activity in particular. Hence, *In vitro* antibacterial activity of crude leaf extracts of these shrubs was tested by disc diffusion method against 9 human pathogenic bacteria *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris Bacillus subtilis, Bacillus cereus, Klebsiella Pneumoniae Salmonella typhi, Enterobacter aerogenes*. Gram-negative bacterial strains were more susceptible to the crude extracts as compare to gram-positive. However, this study revealed maximum growth inhibition and effectiveness was remarkably observed in the extracts of *Vitex negundo* Linn. These results indicate that leaves have a potential broad spectrum antibacterial activity.

KEYWORDS

Vitex negundo Linn, Antibacterial activity, Human pathogens and Disc diffusion.

Author for Correspondence:

S.Anbuselvan,

Annamalai University,

Annamalai Nagar, Tamil Nadu, India.

Email: moks.ra@gmail.com

INTRODUCTION

Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects[1, 2]. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population[3]. The rising incidence in multidrug resistance amongst

pathogenic microbes has further necessitated the need to search for newer antibiotic sources.

Vitex negundo Linn. (Commonly called Chinese chaste tree, Tamil name: *nochhi*) a member of the gamopetalous family Verbenaceae, is an evergreen to semi-evergreen medicinally important shrub or small tree distributed throughout India. The plant is bitter, acrid, thermogenic, expectorant, carminative, digestive, stomachic, anodyne, anti-inflammatory, antiseptic, cephalic, alterant, antipyretic, diuretic, emmenagogue, depurative, rejuvenating, ophthalmic, vulnerary and tonic. The roots used in vitiated conditions of *vata, kapha, jajvara*, cephalalgia, sprains, orchitis, gout, splenohepatomegaly, otorrhoea, inflammations ulcers, cephalagia, otalgia, arthritis, inflammation, dyspepsia, colic, verminosis, flatulence, dysentery, uropathy, wounds, bronchitis, cough, malarial fever, haemorrhoids, dysmenorrhoea, leprosy, dermatopathy, ophthalmopathy and general debility. The bark is used in vitiated conditions of *vata*, odontalgia, verminosis and ophthalmopathy. The flowers used in diarrhoea, cholera, fever, haemorrhages, hepatopathy and cardic disorders[4-6]. The whole plant is having great demand on the market due to its medicinal value. Because of its wide usage and availability, this study was set out to investigate the antimicrobial activity of the plant and phytochemical analysis.

The screening of plant extracts and natural products for antimicrobial activity has revealed the potential of higher plants as a source of new anti-infective agents[7], as well as serving drug discovery from natural products for primary lead compounds. In every developing country it is necessary that the documentation of medicinal plants be treated as a matter of extreme urgency. Hence, the current study was undertaken: To screen the antimicrobial properties of the plant on selective microorganisms and determine the specificity of inhibition on microbes. Phytochemical analysis of plant extracts for their major group of phyto constituents.

MATERIAL AND METHODS

Collection of plant material

Vitex negundo Linn. was collected from patchamalai hill stations.

Preparation of plant extracts

The leaves of *Vitex negundo* were collected and were shade dried, powdered, and extracted in soxhlet apparatus successively with methanol, ethanol, chloroform, and water respectively due to their nature of polarity. After extraction, the hexane and aqueous extracts were filtered through Whatman No.1 filter paper and stored for further use.

From the stock solution different concentrations 25 % (0.5 ml of extract + 1.5 ml of distilled water) 50 % (1.0 ml of extract + 1 ml of distilled water), 75 % (1.5ml of extract + 0.5 ml of distilled water) and 100 % (2 ml of extract only) of the extracts were prepared namely, Ethyl acetate, Chloroform and Aqueous using distilled water.

PHYTOCHEMICAL SCREENINGS

The leaf extracts of *Vitex negundo* were analyzed for the presence of Trepenoids, Flavonoids, Steroids, Anthroquione, Glycosides, Sugars, Alkaloid, Quinones, Phenols, Tannins, Saponins, Protein, lipids and Coumarin according to standard methods[8- 11].

Sterilization of materials

All the glass wares used were washed, dried and sterilized in hot air oven at a temperature of 160°C for 1 h according to the method described[12]. Culture media used were sterilized in an autoclave at a temperature of 121°C for 15 min. The wire loop was also sterilized using spirit lamp.

Isolation and identification of Bacteria

Sample Collection

Food samples were collected from various regions around Tiruchirappalli city using wide mouth sterilized and disinfected container and were transported to the laboratory within an hour after collection. When immediate analyses were not possible, the samples were preserved at 4^0C[13].

Microbial Screening

The samples were serially diluted and spread on to the Sterile Nutrient agar, EMB agar, Mannitol salt agar, Macconkey agar and Pseudomonas isolation agar plate. All the plates were incubated at 37^0C for the 24-48 hrs. Potato dextrose agar plates were incubated at 25^0C for 3-4 days. After incubation, the isolated bacterial colonies were identified by using their morphological characteristic, cell shape by Grams staining, motility and Based on their living cell with standard procedure.

Determination of antimicrobial activity

Disc Diffusion Method

Antimicrobial activity of the leaf extracts was tested using the disc diffusion method[12-14]. Sterile nutrient agar plates were prepared for bacterial strains and inoculated by a spread plate method under aseptic conditions. The filter paper disc of 5 mm diameter (Whatman's No.1 filter paper) was prepared and sterilized. The leaf extracts to be tested were prepared various concentrations of 25 %, 50 %, 75 % and 100 % and were added to each disc of holding capacity 10 microlitre. The sterile impregnated disc with plant extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. Chloramphenicol disc was used as positive control. All the plates were incubated at 37^0C for 24 hours. After incubation, the size (diameter) of the inhibition zones was measured[3].

Determination of MIC and MBC

Minimum inhibitory concentration

About 5ml of Nutrient broth was inoculated with 5µl of test culture and added plant extracts with different concentrations (5-25µl). Chloramphenicol was used as positive control. Incubated the tubes at 37°C for 24 hrs and observed the absorbance at 620nm.

RESULTS AND DISCUSSION

Natural products have been shown to be a tremendous and consistent resource for the development of new drugs[15, 16]. Plants are known to have beneficial therapeutic effects document in traditional Indian System of Medicine[17]. Much work has been done on ethnomedicinal plants in India[18]. It has been suggested that phytochemical extracts from plants holds promises to be used in allopathic medicine as they are potential sources of antiviral, antitumoral and antimicrobial agents[19]. Sometimes plant derived natural compounds have gained attention because of their potential to act as cytotoxic and chemopreventive activity[16]. Various plants have already been proved to possess high antioxidant property containing high amounts of phenolics and flavonoids[15].

The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world[20].

In the present study, the results of phytochemical screening and antibacterial activities were performed with Ethyl acetate, Chloroform and Aqueous extracts using distilled water of the leaf of *Vitex negundo* are shown in Table No.1. The Ethyl acetate extract showed the presence of Trepenoids, Flavonoids, Steroids, Sugars, Quinones, Phenols, Saponins, Protein, Lipids and Coumarin. Glycosides, Sugars, Quinones, Phenols and Tannins were present in Chloroform extract. Glycosides, Sugars, Alkaloid, Tannins and Coumarin were present in aqueous extracts. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. For instance, flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities[21]. Plant steroids are known to be important for their cardiotonic activities and also possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics[22].

Isolation and identification of Bacteria

The Bacteria were identified based on the morphological, motility and biochemical characters as out lined in Bergeys manual of determinative bacteriology. The identified strains and their characteristic feature were represented in Table No.2 and 3.

Anti-Microbial Analysis

In this present investigation compare to Ethyl acetate, Chloroform and Aqueous extracts of the plant

recorded significant zone of inhibition activities against the nine tested bacterial strains are shown in Table No.4,5,6 and 7.

The Ethyl acetate extract of *Vitex negundo* showed the maximum zone of inhibition for *Bacillus cereus* (18 mm), *Bacillus subtilis* (16 mm), and *Enterobacter aerogenes* (12 mm), and The Aqueous extract of *Vitex negundo* showed the minimum zone of inhibition for *Proteus vulgaris* (2 mm) *Staphylococcus aureus* (3 mm) and *Pseudomonas aeruginosa* (4 mm) and the Chloroform extract of *Vitex negundo* exhibited significant result against *Bacillus subtilis, Bacillus cereus* and *Salmonella typhi* ranging from 12 and 16 mm respectively. Dilution screening for antibacterial activity showed a promising effect.

Even though much work has been done on ethnomedicinal plants in India, interest in a large number of traditional natural products has increased of late. Several medicinal plants have been reported to possess antimicrobial, antifungal and other activity has been elucidated by various workers. Phytochemical analysis Table No.8: extracts from *Vitex negundo* plant are potential sources of antimicrobial agents. Several workers have evaluated antibacterial, anti-inflammatory, antiseptic, insecticidal activity of *Vitex negundo* based drugs to meet the health care needs.

In the present work Staphylococcus aureus shows minimum inhibition in aqueous extracts. In the present study, higher activity was found in Ethyl acetate extract of *Vitex negundo* showed high activity against the same strain.

The study was made against 9 Bacterial pathogenic bacteria using the standard disc diffusion method. Varying concentration of all extracts ranging from 5 micro grams to 25 micro grams was tested for antimicrobial activity. Ethyl acetate extract of *Vitex negundo* showed marked activity. Ramya et al, in 2008 had reported that aqueous extract of *T. arjuna* is sensitive towards *E. coli, S. aureus and P. aeruginosa*.

Table No.1: Qualitative analysis of secondary metabolites on *vitex negundo* extracts

S.No	Extracts	Analysis															
		Trepenoids	Flavonoids		Steroids	Anthroquione	Glycosides	Sugars	Alkaloid		Quinones	Phenols	Tannins	Saponins	Protein	Lipids	Coumarin
			T_1	T_2					T_1	T_2							
1	Aqueous	-	-	-	-	-	+	+	-	+	-	-	+	-	-	-	+
2	Ethyl acetate	+	+	+	+	-	-	+	-	-	+	+	-	+	+	+	+
3	Chloroform	-	-	-	-	-	+	+	-	-	+	+	+	-	-	-	-

Table No.2: Characterization of isolated pathogens Based on Morphology

S. No	Character	Organism								
		E. coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Proteus vulgaris*	*Bacillus subtilis*	*Bacillus cereus*	*Klebsiella Pneumoniae*	*Salmonella typhi*	*Enterobacter aerogenes*
1	Gram reaction	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Negative	Negative
2	Morphology	Rod	Rod	Cocci	Rod	Rod	Rod	Rod	Rod	Rod
3	Motility	Motile	Motile	Non-motile	Non-Motile	Motile	Motile	Motile	Motile	Motile
4	Colony Character	Metallic Sheen on EMB	Produces Green Pigment	Ferment mannitol on Mannitol Salt agar	Red pink colonies	Beta heamolytic colonies	heamolytic colonies	Metalilc sheen on macconkey	Red pink colonies	Red pink colonies

Table No.3: Based on biochemical analysis

S. No	Biochemical reaction	Organisms								
		E.coli	*Pseudomonas aerugino*	*Staphylococcus aureus*	*Proteus vulgaris*	*Bacillus subtilis*	*Enterobacter aerogenes*	*Klebsiella Pneumoniae*	*Salmonella typhi*	*Bacillus cereus*
1	Indole	+ ve	- ve	- ve	+ ve	- ve	- ve	- ve	- ve	- ve
2	Methyl red (MR)	+ ve	- ve	+ ve	+ ve	+ ve	- ve	- ve	+ ve	- ve
3	Voges Proskauer (VP)	- ve	- ve	+ ve	+ ve	+ ve	+ ve	+ ve	- ve	+ ve
4	Citrate	- ve	+ ve	- ve	- ve	+ ve	+ ve	+ ve	+ ve	+ ve
5	Urease	- ve	- ve	- ve	+ ve	+ ve	- ve	+ ve	- ve	+ ve
6	Oxidase	- ve	+ ve	- ve	+ ve	+ ve	+ ve	- ve	- ve	- ve
7	Catalase	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	- ve	- ve	+ ve

Table No.4: Antibacterial activity of *Vitex negundo* extracts

S.No	Plant extracts	Zone of inhibition (mm) *E. coli*					
		Positive Control	20 µl	25 µl	30 µl	35 µl	40 µl
1	Aqueous	16	0	0	0	05	07
2	Ethyl acetate	16	0	0	0	6	09
3	Chloroform	16	0	0	0	06	07
Zone of inhibition (mm) *Pseudomonas aeruginosa*							
1	Aqueous	22	0	0	01	03	04
2	Ethyl acetate	22	01	04	06	10	13
3	Chloroform	22	0	01	02	05	07
Zone of inhibition (mm) *Staphylococcus aureus*							
1	Aqueous	20	0	0	01	02	03
2	Ethyl acetate	20	02	6	7	8	10
3	Chloroform	20	0	0	6	8	09
Zone of inhibition (mm) *Proteus vulgaris*							
1	Aqueous	22	0	0	0	01	02
2	Ethyl acetate	22	01	04	06	07	09
3	Chloroform	22	01	04	06	07	08
Zone of inhibition (mm) *Bacillus subtilis*							
1	Aqueous	24	0	04	07	08	10
2	Ethyl acetate	24	02	04	07	09	13
3	Chloroform	24	03	05	09	12	16

Table No.5: Antibacterial activity of *Vitex negundo* extracts

S.No	Plant extracts	Positive Control	20 µl	25 µl	30 µl	35 µl	40 µl
		Zone of inhibition (mm) *Enterobacter aerogenes*					
1	Aqueous	14	0	01	02	03	04
2	Ethyl acetate	14	02	05	08	10	12
3	Chloroform	14	01	02	05	06	09
Zone of inhibition (mm) *Klebsiella Pneumoniae*							
1	Aqueous	16	0	01	02	03	05
2	Ethyl acetate	16	01	02	04	07	09
3	Chloroform	16	0	01	03	05	08
Zone of inhibition (mm) *Salmonella typhi*							
1	Aqueous	*21*	01	02	05	07	08
2	Ethyl acetate	*21*	01	02	05	09	11
3	Chloroform	*21*	02	05	07	10	12
Zone of inhibition (mm) *Bacillus cereus*							
1	Aqueous	*20*	01	02	04	07	09
2	Ethyl acetate	*20*	04	08	11	13	18
3	Chloroform	*20*	03	07	09	10	15

Table No.6: Minimal inhibitory concentration of *Vitex negundo* extracts

S.No	Sample	5 µl	10 µl	15 µl	20 µl	25µl
		Absorbance at 620nm *E. coli*				
1	Positive Control	0.056	0.056	0.055	0.055	0.054
2	Aqueous Extract	0.765	0.748	0.745	0.740	0.735
3	Ethyl acetate Extract	0.655	0.581	0.556	0.528	0.454
4	Chloroform Extract	0.963	0.864	0.755	0.707	0.690
Absorbance at 620nm *Pseudomonas spp*						
1	Positive Control	0.039	0.038	0.037	0.034	0.030
2	Aqueous Extract	0.921	0.869	0.841	0.810	0.780
3	Ethyl acetate Extract	0.530	0.470	0.460	0.390	0.364
4	Chloroform Extract	0.890	0.860	0.835	0.773	0.699
Absorbance at 620nm *Staphylococcus spp*						
1	Positive Control	0.057	0.056	0.056	0.056	0.056
2	Aqueous Extract	0.843	0.812	0.800	0.796	0.735
3	Ethyl acetate Extract	0.420	0.370	0.310	0.270	0.221
4	Chloroform Extract	0.810	0.790	0.770	0.729	0.715
Absorbance at 620nm *Proteus vulgaris*						
1	Positive Control	0.065	0.040	0.035	0.025	0.015
2	Aqueous Extract	0.870	0.839	0.820	0.786	0.639
3	Ethyl acetate Extract	0.590	0.470	0.410	0.385	0.317
4	Chloroform Extract	0.660	0.649	0.637	0.619	0.550
Absorbance at 620nm *Bacillus subtilis*						
1	Positive Control	0.080	0.070	0.054	0.052	0.052
2	Aqueous Extract	0.970	0.939	0.920	0.886	0.839
3	Ethyl acetate Extract	0.490	0.470	0.430	0.420	0.337
4	Chloroform Extract	0.880	0.869	0.857	0.829	0.780

Table No.7: Minimal inhibitory concentration of *Vitex negundo* extracts

S.No	Sample	5 µl	10 µl	15 µl	20 µl	25µl
		Absorbance at 620nm *Enterobacter aerogenes*				
1	Positive Control	0.086	0.075	0.065	0.055	0.054
2	Aqueous Extract	0.565	0.548	0.545	0.540	0.535
3	Ethyl acetate Extract	0.655	0.581	0.456	0.428	0.354
4	Chloroform Extract	0.794	0.725	0.655	0.507	0.480
Absorbance at 620nm *Klebsiella Pneumoniae*						
1	Positive Control	0.053	0.048	0.042	0.038	0.030
2	Aqueous Extract	0.821	0.769	0.710	0.680	0.630
3	Ethyl acetate Extract	0.530	0.470	0.460	0.390	0.364
4	Chloroform Extract	0.897	0.850	0.815	0.789	0.721
Absorbance at 620nm *Salmonella typhi*						
1	Positive Control	0.080	0.060	0.044	0.032	0.022
2	Aqueous Extract	0.939	0.919	0.890	0.866	0.819
3	Ethyl acetate Extract	0.490	0.470	0.430	0.420	0.377
4	Chloroform Extract	0.680	0.629	0.597	0.529	0.490
Absorbance at 620nm *Bacillus cereus*						
1	Positive Control	0.080	0.070	0.054	0.052	0.052
2	Aqueous Extract	0.870	0.839	0.810	0.786	0.739
3	Ethyl acetate Extract	0.590	0.570	0.510	0.480	0.415
4	Chloroform Extract	0.795	0.758	0.743	0.712	0.684

Table No.8: Qualitative Phytochemical tests

S.No	Experiment	Observation	Inference
1	Test solution + minimum amount of chloroform + 3 drops of conc. H_2SO_4 (Libermann Burchard test)	Purple colour changing to blue or green	Presence of Steroids
2	Test solution + piece of tin + 3 drops of Thionyl chloride	Violet or purple colour	Presence of Triterpenoids
3	Test solution + Molish's reagent	Purple colour	Presence of reducing sugars
4	Test solution +10% NaOH solution and heated/	Solution turned brown on heating	Presence of Carbohydrates
5	Test solution shaken with 2N HCL. Aqueous layers formed decanted to Mayers reagent are added	White turbidity or precipitate	Presence of Alkaloid
6	Alcoholic solution of test solution+ one drop of Ferric chloride	Intense colour	Presence of Compounds
7	Test solution + water, shaken well	Foamy lather	Presence of Saponins
8	Test solution + conc.HNO_3 excess Ammonia	Reddish orange precipitate	Presence of Xantho proteins
9	Water solution portion of the extracts treated with basic lead acetate solution	White precipitate	Presence of Tannins
10	Test solution+ Magnesium powder and treated with conc.HCL and heated. cool the test tube under the running water	Orange colour	Presence of Flavonoids

CONCLUSION

The studies reveal that the Ethyl acetate is better than that of Chloroform and Aqueous extracts of *Vitex negundo* in respect to their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities.

Phytochemical Analysis

The qualitative analysis of phytochemical screening of *Vitex negundo* is shown in Table No.8. Aqueous extract of leaf Glycosides, Sugars, Alkaloid, Tannins, Saponins and Coumarin was present. Ethyl acetate extract of leaf Trepenoids, Flavonoids, Steroids, Sugars, Quinones, Phenols, Saponins, and Coumarin was present. Chloroform extract of leaf Glycosides, Sugars, Quinones, Phenols, and Tannins was present.

Larger quantities in core-wood in comparison with that of bark. Hexane extract of core wood showed the presence of triterpenoids while bark extract did not show any trace of triterpenoids. Both the extracts were negative for cardiac glycosides, acids, alkaloids, sugars and proteins. The data obtained was consistent with Dymock et al., 1891, Row et al., 1970 and Sharma et al., 1982, who had mentioned the presence of tannins, flavonoids, and terpenoids in the bark.

Trepenoids, Flavonoids, Steroids, Anthroquione, Glycosides, Sugars, Alkaloid, Quinones, Phenols, Tannins, Saponins, Coumarin.

Anti-Microbial Analysis: Varying concentration of all extracts ranging from 10 micro grams to 1000 micro grams was tested for antimicrobial activity. None of the extracts showed marked activity. Ramya et al, in 2008 had reported that aqueous extract of *T. arjuna* is sensitive towards *E. coli, S. aureus and P. aeruginosa*.

Phytochemical analysis confirms the presence of Trepenoids, Flavonoids, Steroids, Anthroquione, Glycosides, Sugars, Alkaloid, Quinones, Phenols, Tannins, Saponins, Coumarin. We recommend further research on this plant for possible isolation characterization of the various chemical active substances.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Bibitha B, Jisha VK, Salitha CV, Mohan S, Valsa AK. Antibacterial activity of different plant extracts, Short Communication, *Indian J Microbiol*, 42(6), 2002, 361-363.
2. Maghrani M, Zeggwah N, Michel J and Eddouks M. Antihypertensive effect of *Lepidium sativum* in spontaeneously hypertensive rats, *J Ethnopharm*, 102(1-2), 2005, 193-197.
3. Doughari JH. Antimicrobial Activity of *Tamarindus indica* Linn, *Trop J Pharm Res*, 5 (2), 2006, 597-603.
4. Johnson Ted T and Christine case. Laboratory experiments in microbiology, *The Benjamin cummings publishing co. Ine California*, 61(1), 1986, 34-35.
5. Johnson M, Sonali Das, Yasmin N and Rajasekara Pandian M. Micropropagation Studies on *Vitex negundo* L. - A Medicinally Important Plant, *Ethnobotanical Leaflets*, 12(4), 2008, 1-4.
6. Warrier P K, Nambiar V P K and Raman Kutty. *Vitex negundo* L. In Indian medicinal plants, *Orient Longmen Ltd., Madras India*, 4, 1995, 371.
7. Mitscher L A, Drake S, Goliapudi S R and Okwute S K. A modern look at Folroricuse of anti-infective agents, *Journal of natural products*, 50(6), 1987, 1025-1040.
8. Sai Ramesh A J, Godwin Christopher C R, Setty V. Thankamani. Comparative study on the yield ratio and bioactive compounds of *Terminalia arjuna* bark and core-wood, *Journal of Pharmacy Research*, 3(6), 2010, 1420-1422.
9. Harbone J B. Role of secondary metabolities in chemical defence mechanisms in plants.

Bioactive compounds from plants, Ciba foundation symposium 154. wiley chichester, 185, 1990, 126-139.
10. Harbone S V. Biochemistry of plant phenolies, *Recent Advances in Phytochemistry*, 12(15-19), 1973, 760-764.
11. Trease G E and Evans W C. Pharmacognosy. *Brailliar Tiridel and Macmillian Publishers,* London, 11th edition, 1989.
12. Adibe NC, Eze EI. General Laboratory Techniques for Tertiary Institutions, *Mike Social Publishers, Enugu, Nigeria*, 15(3-4), 2004, 301-304.
13. Aida P, Rosa V, Blamea F, Tomas A, Salvador C. Paraguyan plants used in traditional medicine, Short communicaton, *J Ethnopharm*, 13(16), 2001, 93-98.
14. Peach K and Tracey M V. Modern methods of plant analysis, *Springer- verlag, Berlin*, 2, 1950, 645.
15. Kalaivani T, Mathew L. Phytochemistry and Free radical scavenging activities of Oroxylum indicum, *Environment and We an International Journal of Science and Technology*, 4, 2009, 45-52.
16. Kalaivani T, Rajasekaran C, Suthindhiran K, Mathew L. Free radical scavenging, cytotoxic and hemolytic activities from leaves of *Acacia nilotica* (L.) Wild. ex. Delile subsp. indica (Benth.) Brenan, Evidence based Complementary alternative medicine, *Food and Chemical Toxicology*, 48(1), 2010, 298-305.
17. Desai Nivas, Gaikwad D K and Chavan P D. Antiradical activity of medicinally important *Morinda pubescens* Fruits, *International Journal of Pharma and Bio Sciences,* 1(3), 2010, 1-4.
18. Vinoth Raja R, Ramanathan T and Savitha S. Studies on wound healing property of coastal medicinal plants, *J Biosci Tech,* 1(1), 2009, 39-44.
19. Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected indian medicinal flora, *Turk Journal of Biology*, 29(1), 2005, 41-47.
20. Reddy PS, Jamil K, Madhusudhan P. Antibacterial activity of isolates from *Piper longum* and *Taxus baccata*, *J. Pharmaceutical Biol*, 39(3), 2001, 236-238.
21. Aiyelaagbe O O and Osamudiamen P M. Phytochemical screening for active compounds in *Mangifera indica* Plant, *Sci. Res*, 2(1), 2009, 11-13.
22. Callow R K. Steroids, *Proc. Royal, Soc London Series A*, 157(890), 1936, 194-212.
23. Harbone J B. Phytochemical Methods, *London: Chapman and Hill*, 197.

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