ISSN : 2249-5746

International Journal of Ayurvedic and Herbal Medicine 3:5 (2013) 1373–1383

Journal homepage: http://www.interscience.org.uk

Tridax pombens: A source for antimicrobial activity

Priyadarshini. D. S and Priya Iyer

P.G. and Research Department of Biotechnology

Women`s Christian College, Chennai-600006.

ASTRACT:

Tridax Proumbens is a species following plant in the daisy family. Its common names include coat buttons and Tridax daisy. Callus and suspension culture was obtained using different explants.The different solvent extracts of Tridax plant and callus was checked for antimicrobial activity against Escherchia coli, Staphylococcus aureus, Pseudomonas aerogenosa, Aspergillus niger and Penicillium chrysogenum. The plant was also tested for the presence of different phytochemicals.

KEY WORDS: *Tridax* Proumbens, *Escherchia coli*, *Staphylococcus aureus*, *Pseudomonas aerogenosa*, *Aspergillus niger* and *Penicillium chrysogenum*

INTRODUCTION

Tridax proumbens is best know as a wide spread weed and pest plant. It is native to the tropical Americas but it has been introduced to tropical, subtropical, and mild temperature regions world wide. The plant bears daisy like yellow – centered white or yellow flowers with three – toothed ray florets. The extracts of *Tridax procumbens* have been reportedn to have various pharmacological effects,antimicrobial activity against both gram-positive and Gram negative bacteria. The extracts of *T.procumbens* have been reported to have various pharmacological effects including antimicrobial activity, wound healing property and immunomodulatory activity on the experimental animals **(Babu *et al.*, 2003; Oladunmoye, 2006;Udopa *et al*;1991, Diwan *et al*; 1982).**

MATERIALS AND METHODS

Microorganisms: Culture of *Staphylococcus aereus, Pseudomonas aerogenosa, Escherichia Coli, Penicillium chrysogenum* and *Aspergillus niger* was procured from IMTech, Chandigarh.

Plant materials and solvent extraction: The aerial parts of *Tridax procumbens* were collected and washed clearly. These materials were shade-dried and coarsely powered. These coarse powders were then subjected to successive extractions by various solvents such as ethanol, chloroform and Acetone. The collected extracts were then taken up for further investigations. About 10 grams powdered materials were mixed with various solvents (ethanol, Chloroform and acetone) in a conical flask. The flask was kept on shaker for 24 hours for extraction and after 24 hours the materials are centrifuged at 9500 rpm for 15 minutes. The supernatant were collected and allowed to evaporation of solvent. The extract was stored in the refrigerator.

For aqueous extraction, 10 g of air-dried powder was mixed with 100 ml distilled water and kept at room temperature for 48 h. It was then filtered through muslin cloth and centrifuged at 5000 *g* for 10 min. The supernatant was collected and stored at 4^{o}C. About 5 different combination of the plant extract were prepared by mixing ethanol, chloroform and acetone extract in different ratio. The ratio of 1:1:1, 2:2:2, 1:2:2, 2:1:2, 2:2:1, The mixture 1:1:1 contain 0.5 ml of Ethanol : Chloroform : Acetone, and 2:2:2 contain 1 ml of Ethanol : Chloroform : Acetone.These extract were separately kept in eppendroff of stored in the refrigerator.

Inhibition of various extract on different microorganisms: The organism were spread on Muller Hinton medium and antimicrobial activity of the extracts was carried out by disc diffusion and agar gel diffusion method.Following application of the different extract (ethanol, chloroform and acetone) all the plates were incubated for 24 hours at 37^{o} C. After 24 hours, the plates were taken out and the zone of inhibition was measured in mm

Initiation of callus and cell suspension culture: Sterile MS media was used for callus initiation. Indole – 3 – acetic acid (1mg/ml) 2,4 – Dichlorophenoxyacetic acid (1mg/ml) and Naphthalene acetic acid (1mg/ml) were the hormones used which were sterilised using memberane filter and then added to sterile media. Leaf, shot bud and stem of the plant was surface sterilised with 70% ethanol and 1% sodium hypochlorite before inoculation into sterile MS media. For cell suspension culture callus or plant explants were allowed to grow in liquid MS media.

Phytochemical analysis: The extracts of the plant and callus were used tannins estimation. The extracts were tested for flavonoids, phlobatanins, saponins, steroids, terpenoids and cardiac glycosides.

RESULT AND DISCUSSION

Inhibition of various extract on different microorganisms: The ethanolic extract show zone of inhibition in disc diffusion and agar gel diffusion method after incubation of plates for 24 hours at 37^{o} c.As per the inhibition results, *Escherichia coli* showed maximum zone of inhibition in ethanol extract compared to actone and chloroform extract showed same level of inhibition for *Escherichia coli*. *Staphylococcus aereus* showed maximum level of inhibition in ethanol and chloroform extract .The minimum level of inhibition was observed in the plate with acetone extract. *Pseudomonas aerogenosa* showed maximum level of inhibition in chloroform extract compared to ethanol and acetone extract. *Aspergillus niger* showed maximum and same level of inhibition in acetone and chloroform extract. The minimum level of inhibition was observed in ethanol extract. *Penicellium chrysogenum* showd maximum level of inhibition in acetone extract. They showed same level of inhibition in ethanol and chloroform extract.The antimicirobial assay of aqueous and methanolic extract was performed by two methods (Table I). The agar disc diffusion method (Bauer et al., 1966) and agar well diffusion method (Perez et al., 1990) The n-hexane estract of the flowers showed activity against *Escherichia coli.* The same extract of the whole areal plant was active against *Mycobacterium smegmatis, Escherichia coli, Salmonella group c and Salmonella Parathypi*. The ethyl acetate extract of flower was active against species. The areal part extract also showed activity only against *Mycobacteria smegmatis* and *Staphylococcus aureus* while aqueous extract showed no antimicrobial activity. None of the test was active against yeast and *Candida albicans* (Taddei A et al., 2000)

Table I: ANTIMICROBIAL ACTIVITY OF TRIDAX PROCUMBENS VARIOUS EXTRACT ON MICROORGANISMS

Microogranisms	Zone of inhibition for ethanol extract (mm)	Zone of inhibition for acetone extract (mm)	Zone of inhibition for chloroform extract (mm)
Escherichia coli	16	14	14
Staphylococcus aureus	13	9	13
Pseudomonas aerogeosa	11	10	16
Aspergillus niger	11	15	15
Penicillium chrysogenum	13	14	13

Inhibition action of Chloroform extract by disc diffusion method: The chloroform extract showed zone of inhibition in the disc diffusion method. When compared to get diffusion method this disc diffusion method gives low level of inhibition activity.(TableII) Of this *Staphylococcus aureus* and *Penicillium chrysogenum* shows maximum level of inhibition and all the other three organisms shows very low level of inhibition. Antimicrobials of plant origin have enormous therapeutic Potential and have been used since times and memorial *Tridax procumbens* is known for its wound healing activity. Whole plants is made into paste and applied on fresh cuts (Dhar et al, 2008). The antibacterial and antifungal activity of chloroform acetone, methanol and aqueous extract of andrographis echiodes at different concentrations against seven strains of bacteria were investigated (Umadevi et al,2003)

TABLE II: INHIBITION OF EFFECT OF CHLOROFORM EXTRACT BY DISC DIFFUSSION METHOD

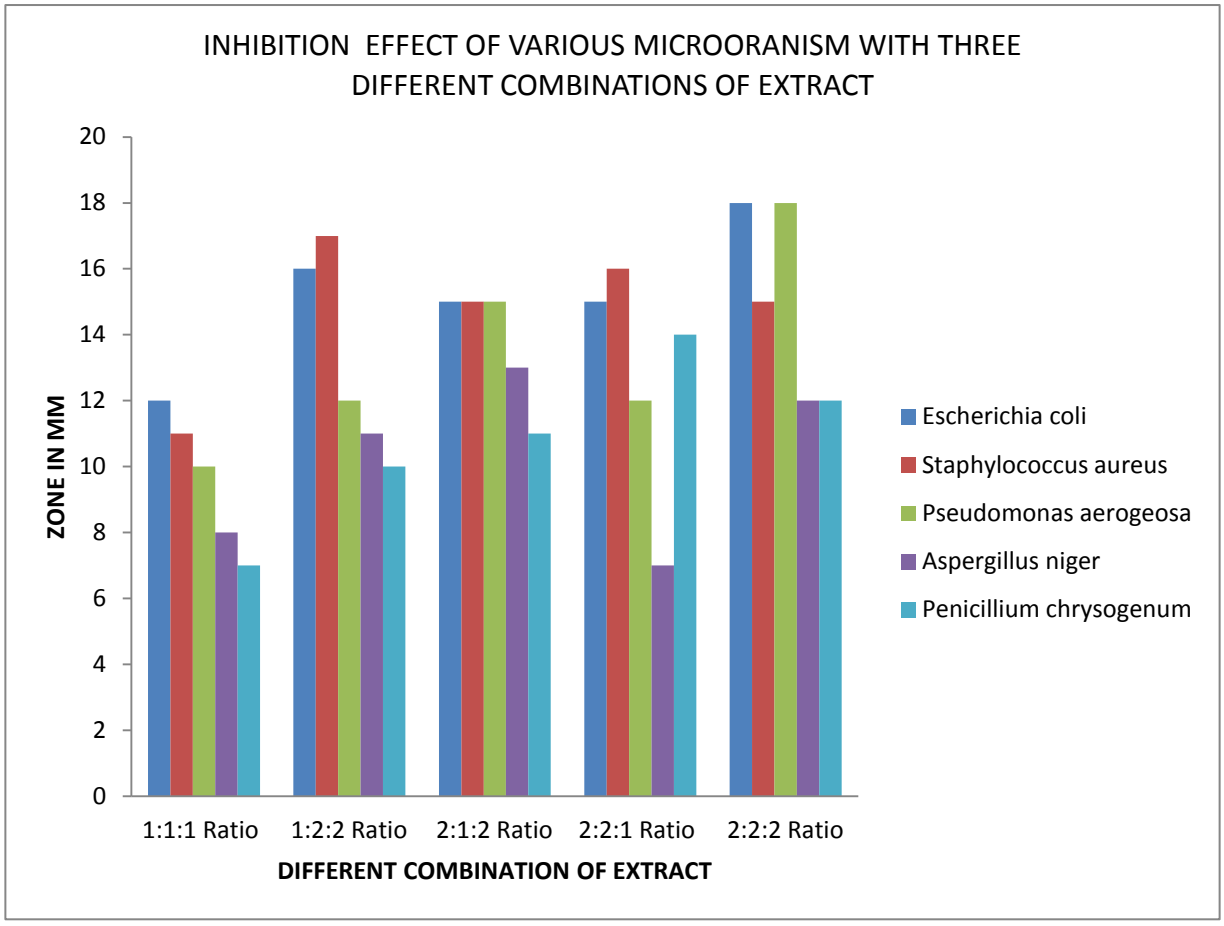
Microogranisms	Zone of inhibition in mm
Escherichia coli	5
Staphylococcus aureus	11
Pseudomonas aerogeosa	9
Aspergillus niger	8

Penicillium chrysogenum	10

Inhibitory activity of aqueous extract:The aqueous extract didn't show any zone of inhibition for any organism in disc diffusion method after incubation for 24 hours .The methanol extract of *Tridax procumbens* showed significant activity against coagulase positive *Staphylococcus aerues* . But only least antimicrobial activity was observed on other selected bacterial strains. The aqoues extracts of *Tridax procumbens*showed no pronounced antimicrobial activity against *Staphylococcus uberis* and *Klebseilla Pneumonia* (Palaniswamy et al.,2008).The antimicrobial assay aqueous and methanolic extract was performed by two methods. The agar disc diffusion method and agar well diffusion method (Perez et al., 1990).

Inhibitory action of combination of Ethanol, Chloroform and Acetone extract: The various combinations of three alcoholic extract, ethanol, chloroform and acetone extract shows different zone of inhibition is various ratios (Graph I).The maximum level of inhibition was observed in 2:2:2 combination ratio for Escherichia coli and same level of inhibition in 2:1:2 and 2:2:1 ratio.All the organisms except *Aspergillus niger* shows minimum level of inhibition in 1:1:1 ratio . The *Aspergillus niger* show minimum level of inhibition in 2:2:1 ratio.The same level of inhibition were observed in 2:1:2 ratio of *Pseudomonas aerogenosa , Escherichia coli and Staphylococcus aureus. Aspergillus niger* shows maximum level of inhibition in this ratio.*Staphylococcus aureus* shows maximum level of inhibition in 1:2:2 ratio and same level of inhibition in 2:1:2 and 2:2:2 ratio.*Pseudomonas aerogenosa* shows maximum level of inhibition in 2:2:2 ratio and same level of inhibition in 1:2:2 and 2:2:1 ratio.*Pseudomonas aerogenosa* shows maximum level of inhibition in 2:2:1 ratio.

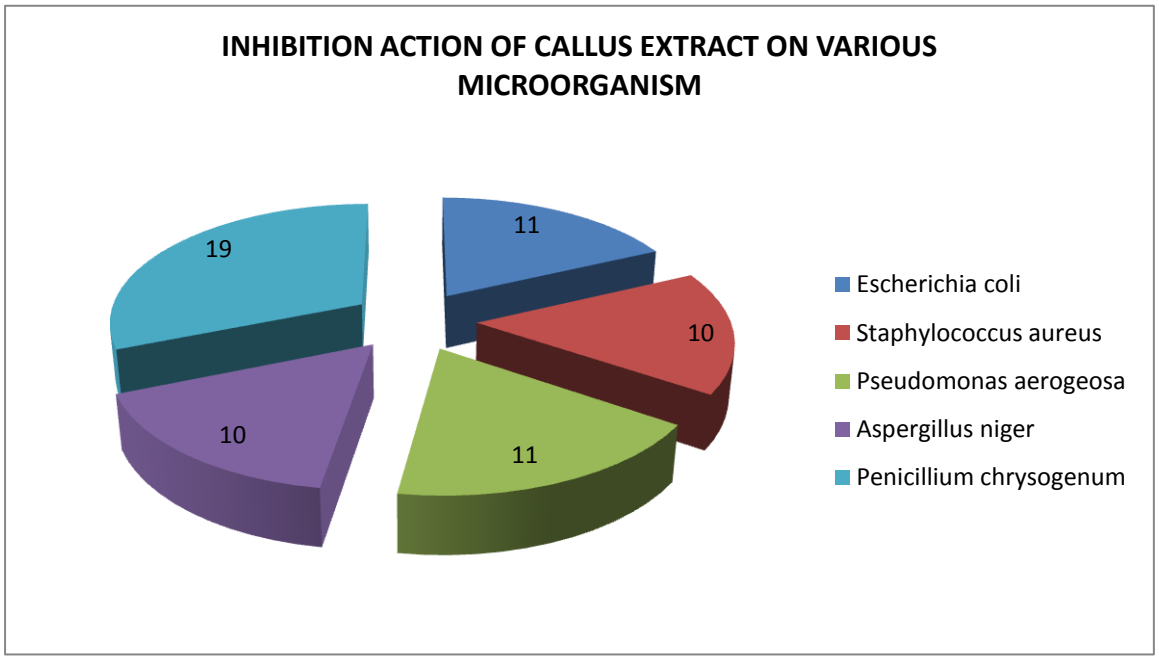
Graph I



Tissue culture of Tridax Proumbens: The inoculation of explants on the MS media with vitamins and sugar yield the callus from leaf and stem, after 2 weeks of incubation. The hormone concentrations that were tried for this experiments were 5µl, 10µl, 50µl, 100µl and 150µl of 2, 4D. IAA, NAA. No rooting and shooting was formed even after 8 weeks. But inoculation of callus obtained from MS media with vitamins and sugar on MS media with 2, 4D shows root formation.The plant leaves were macerated into small pieces and inoculated in MS media without agar. Then they are allowed to incubate for 2 weeks. The cells suspension was viewed under inverted microscope periodically. Vascular bundles and cluster of cells observed under microscope.

Inhibitory action of callus extract on different microorganisms: The callus obtained from tissue culture of *Tridax procumbens* were extracted with ethanol. This extract was tested for their antimicrobial activity on different microorganism. All the plates were incubated for 24 hours at 37 c after inoculation. After 24 hours the plates were taken out and the zone of inhibition was measured in mm (Graph II).The maximum level of inhibition were obserbed for *Penicillium chrysogenum. Staphylococcus aureus and Aspregillus niger* shows minimum level of inhibition and *Escherichia coli* and *Pseudomonas aerogenosa* shows same level of inhibition activity.

Graph II



After inoculation of all the culture in the plates the solvents like ethanol, chloroform and acetone were poured into the well. Then the plates were incubated for 24 hours at 37°C. No zone of inhibition were observed in all the plates.

Inhibitory action of flavanoid on microorganisms: The flavanoids of this *Tridax procumbens* have antimicrobial activity. The flavaniods extracted from this plant were tested for their activity. Following application of flavanoids in the well, all the plates were incubated for 24 hours at 37°C. After 24 hours, the plates were taken out and the zone of inhibition was measured in mm. (Table III).For flavanoid extract *Penicilliun chrysogenum* showed maximum level of inhibition and *Aspergillus niger* showed minimum level of inhibition.All the other three organisms also shows high level of inhibition.Three extract of *Tridax procumbens* viz bound flavanoids of root,ledf and flowers was found to be bactericidal against Pseudomonas mirabilis and bound flavonoids of leaf were found to be fungicidal against *Candida albicans.* Gram positive bacteria , *Staphylococcus aureus* was the second most susceptible organism after *Candida albicans* which supported the finding that plant extract are usually more active in Gram positive bacteria than Gram negative(Lin et al 1999).The phytochemical screening revealed the presence of alkaloids, tannins,saponin, steroids, terpenoid and flavonoids. Most of the secondary metabolites is higher than non-polar metabolites in leaves of these species (Jamine et al 2007).

The flavanoid sample was allowed to run in paper chromatography. After the sample were run to ¾ of the paper they were allowed to dry and sprayed with ammonium hydroxide. A yellow spot were observed on the paper indicate the presence of flavanoid in the sample.

Table III INHIBITORY ACTION OF FLAVANOIDS EXTRACT ON VARIOUS MICROORGANISMS

Microogranisms	Zone of inhibition in mm
Escherichia coli	*16*
Staphylococcus aureus	*17*
Pseudomonas aerogenosa	*15*
Aspergillus niger	*11*
Penicillium chrysogenum	*26*

Graph: 4

Phytochemical screening Tests: The result for this test were indicate the presence or absence of the phytochemicals (TableIV). Steroids and phlobatannins were found to be present in all the plants. It has been found that some of these investigated plants contained steroidal compounds. It should be noted that steroidal compounds are of important and interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001)

Medicinal plants are of great importance to the health of individuals and communities. The medical value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds(Hill, 19520)

Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to food meant for pregnant and nursing mothers for medicinal purposes (Okwn, 1999,2001)

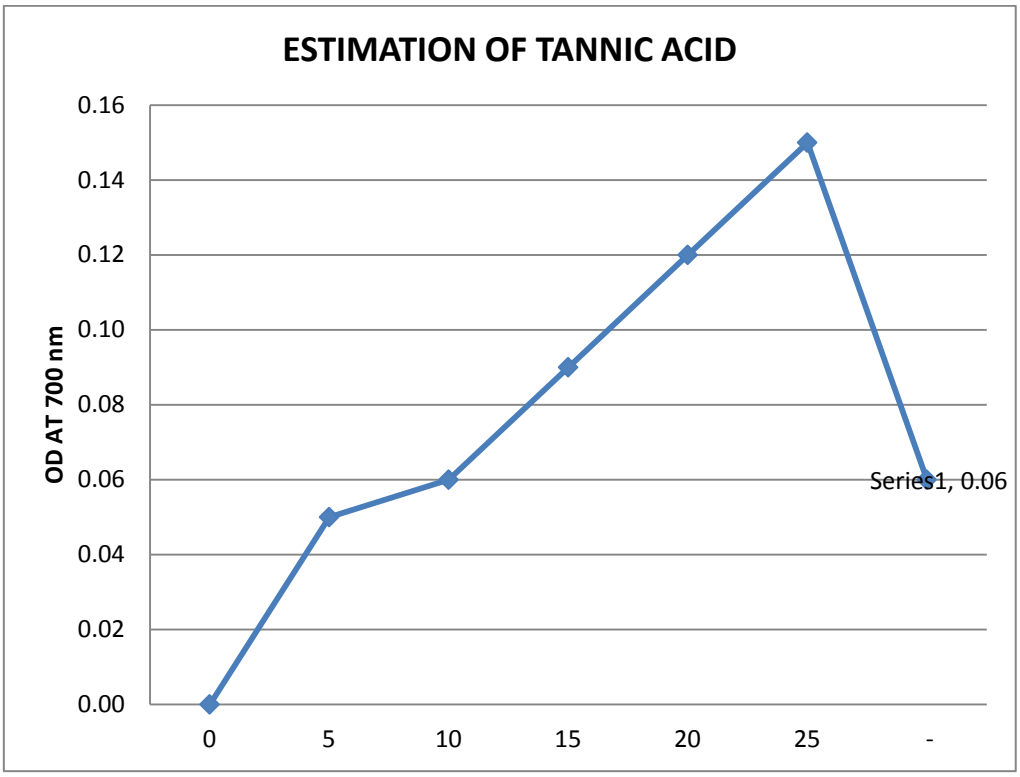
on the addition of 0.1 FECL2 solution on sample indicate the presence of tannins.

TABLE IV: PHYTOCHEMICAL SCREENING TEST

PHYTOCHEMICALS	TRIDAX PROCUMBENS
Tannin	+
Saponin	+
Steroid	-
Phlobatannin	-
Terpenoid	-
Flavonoid	+
Cardic glycoside	-

Estimation of Tannic acid: The amount of tannic acid was estimated by Folin-Denis method Graph III.

CONCLUSION: The plant studied here can be seen as a potential source of useful drugs. Further studies are going on this plant in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. The antimicrobial activities of these plants for treatment of the diseases as claimed by traditional healers are also being investigated.



BIBLIOGRAPHY

Taddei A, Rosas-RomeroA,Phytomedicine 2000 JUN;7(3)235-8; None of the test was active against *Yeast* and *Candida albicans.*

Lin J.Opake AR Geheeb –Keller M, Hutchings AD, Terbianche SE, Jaeger AK 1999, Preliminary screening of some traditional Zulumedicinal plants for anti-inflammatory and antimicrobial activity.**Ethanopharmacol 68;267-274**

Dhara U Singh UK, Aminuddin 2003 Ethanobotany of Bhuyans and Juangs of Orrisa In Singh UK ,Govil JN, Hashmi S, SinghG,eds,Recent progress in medicinal plants,Vol.7.USA,Studium press LLC,P.200

Taddel A, Rosas Romero AJ. (2000): Bioactivitystudies of extracts from *Tridax procumbens*.Phytomedicine, Jun 7: 3 235-8

Udopa SL, Udopa AL and Lalkarni DR (1991): Influence of *Tridax procumbens* on lysl oxidase activity and wound healing. Planta Med Aug 57: 4, 325-7

Yadawa RN Saurabh K (1998): A new flavone glycoside: 5,7,4 –Trihydraxy – 6, 3 – dimethasey Falavone 5-0 alpha –L- rhamnopyramoside from the leaves of *Tridax procumbens* Liun. J. Asian Nat. Prod. Res. 1:2,147-52

Ali,M., Rawinder,E., Ramachamdram, R., 2001. A new flavonoid from the aerial parts of *Tridax procumbens*. Fitoterapia. 72: 313-315

Babu, G., Sanjeeva., Bairy, K.L., 2003. Effect of *Tridax Procumbens* on burn wound healing. Indian Drugs. 40: 488-491

Diwan, P.V., Tilloo, L.D., Kulkarni, D., 1982. Influence of *Tridax procumbens* on wound healing. Indian J. Med Res. 75: 450-454

Jamine. R, Daisy.P and Selvekumar. B.N., 2007. *In vitro* Efficacy of Flavonoids from *Eugenia jambolana* Seeds Against ESL-Producing Multidrug-Resistant Enteric Bacteria. Research Journal of Microbiology. 2 (4):369–374.

Prasad, N. R., Viswanathan.S., Renuka Devi,J., Vijayashree Nayak., Sweth,V.C., Archana Parathasarathy, N and Johanna Rajkumar., 2008. Preliminary phytochemical screening and antimicrobial activity of *Samanea saman* . Journal of Medicinal Plants Research. 2: 268-270.

Oladunmoye,M.K., 2006. Immunomodulatory effects of Ethanolic Extract *of Tridax procumbens* on Swiss Albino Rats Orogastrically Dosed with *Pseudomonas aeruginosa* (NOB 950). Trends in Medical Research. 1: 122-126.

Suseela, L., Sarsvathy, A., Brindha, P., 2002. Pharmacognostic studies on *Tridax procumbens* L.(Asteraceae). Journal of Phytological Research. 15: 141-147.

Trease, G.S., and Evans, H.C., 1978. Textbook of Pharmacognosy. 9 th edition. Bailiar Zindall And Co., London.

Udopa, S.L., Udopa, A.L and Lalkarni, D.R., 1991. Influence of *Tridax procumbens* on lysl oxidase activity and wound healing. Planta Med. 57: 325-7

Yadawa, R.N., Saurabh, K., 1998. A new flavones glycoside: 5,7,4 –Trihydraxy – 6, 3 – dimethasey Falavone 5-0 alpha –L- rhamnopyramoside from the leaves of *Tridax procumbens* Liun. J. Asian Nat. Prod. Res. 1:147-52.

Egunjiobi JK (1969). Some common weeds of West Africa. Bull. Res. Div. Ministry of Agric. Natural Resources Westenn State,ibadan, Nigeria.

VERMA, R.K.; GUPTHA, M.M. Lipid constituents of Tridax procumbens. Phytochemistry.Oxford, v.27, n.2, p.459-463, Feb.1988.

NASEEM, M.; JHA,K.K Differentiation and regeneration in Cleome viscose leaves cultured invitro.Egyptian Journal of botany, Cairo,v.34, n.1, p.37-47, Set. 1994.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disc method . Am. J. Clin. Pathol.45:493-496.

Planiswamy, M., Pradeep, B.V., Sathya,R., Angayarkanni,J.,2008. In vitro anti-plasmodial activity of Trigonellla foenum- graecum L. ecam 2008; dio:10.1093/ecam/nen030;1-5.

Umadevi S, Mohanta GP, Chelladurai v, Manna PK ,Manalavan R**.** Antimicrobial and antifungal activity of Andrographis echiodes .J Natural Remed 2003; 3:185-188.

Hill AF (1952).Economic Botany. A Text book of useful plants and plant products. 2nd edn. McGarw- Hill Book Company Inc,New York.

Okwu DE (1999) . Flavouring properties of spices on cassava Fufu,Afr. J.Roots Tuber Crops 3(2):19-21.

Oker DE (2001).Evaluation of the chemical composition of indigenous spices and flavoring Agents . Global J .Pure Appl.Scl. 7(3):455-459.