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Microbiology

A STUDY ON EFFICACY OF METHANOLIC EXTRACT OF GARCINIA GUMMI-GUTTA DRIED FRUITS

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ABSTRACT Plant extracts has been used to treat for contagious disease from very old time in outmoded medical systems. The remedial plants for the treatments for various diseases may lie in the antioxidant and antimicrobial effect of phytochemicals. Due to the development of confrontation in pathogenic microorganisms to antibiotics used in modern therapeutic science, there is a growing awareness towards plant extracts as a source of new antimicrobial drug discoveries. The ripened fruit of Garcinia gummi-gutta were collected from various part of Wayanad, India and the present study was carried out to assess various properties of the methanolic extract of Garciniagummi-gutta. The methanolic extract of Garcinia gummi-gutta shows the maximum antibacterial activity against Streptococcus pyogens and also against Staphylococcus aureus It also has the antioxidant property.. The extract shows the ovicidal against Haemonchuscontortus and also exihibit cytotoxic acitivity against Artemiasalina. The result acquired from this study suggests that the identified phytochemical compounds may be the bioactive constituents and this fruit is proving to be a valuable reservoir of bioactive compounds of substantial medicinal merit which can be commercially exploited.

KEYWORDS : antioxidant phytochemicals, cytotoxic, Garciniagummi-gutta.

I. INTRODUCTION

World Health Organisation (WHO) has suggested that medicinal plants contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs. The plant *Garcinia gummi-gutta* belongs to the family *Guttiferae {Clusiaceae}*. It is a wild sub tropical and tropical plant. Dmitrityobolskiy *et al*., (2009) observed that the plant is commercially important as their fruit extracts are used for various treatments such as astringent, demulcent, rheumatism and bowel complaints.

The genus *Garcinia* has been involved in Ayurvedic preparations to medicate various patho-physiological disorders. The main component of the fruits is hydroxyl citric acid and is used in anti obesity drugs. The fermented fruit extract is used as a souring agent in parts of Kodagu and Kerala. In justifying its endemic property to Western Ghats, it is evident to do a complete analysis on these plants which will help justifying which region plants are qualitatively better. These plants in various regions of Western Ghats will show variations in their chemical content as they are influenced by climatic and edaphic features. *Garcinia gummi-gutta,* a common medicinal plant, has been used in the past to treat respiratory infections such as sore throat and cough. The phytochemical constituents include biflavonoid, xanthone and benzophenones and the principle acid in the fruit and rind is hydroxy citric acid. This acid has been found to suppress fatty acid synthesis, lipogenesis, food intake and to promote glycogenesis, while inducing weight loss. The sun-dried rind of the fruit is astringent, antiseptic and purgative. The efficacy of *G. gummi-gutta* on the lowering of fatty acid composition in mammals has already been reported.

In this paper we reviewed the traditional and medicinal properties of the fruit rind and seeds of *Garcinia gummi-gutta.* In the west coast of South India, *Garcinia gummi-gutta* is commonly known as "Malabar Tamarind". Mostly these species are forest products. Karnataka forest publication 2011 has reported these plants as forest trees with medicinal aspects. Hence breeding of these trees has to be boosted. The fruit trade is at global level. Biomolecules from flora belong to a diversity of substance of polar compounds and non polar compounds which is readily soluble in methanol. Thus mostly methanol is used for extract in the present study, the antimicrobial activity, MIC, phytochemical screening, anti-oxidant properties, In-vitro ovicidal activity and the potential cytotoxic activity of methanolic extract of *Garcinia gummi-gutta* dried fruit *was analysed.*

I. MATERIALS AND METHODS

A. Collection of sample and Methanolic extract of Garcinia gummi-gutta

The ripe fruit of *Garcinia gummi-gutta* were collected from various part of Wayanad ,India . The fruit sample of 1 Kg were collected in polythene bags and taken to the laboratory. The fruit were washed with clean sterile water. Then the fruits then shade dried until all the water molecules evaporated. After drying the fruit were ground well using mechanical blender into fine powder and then transferred into air tight container. Dried fruit sample were weighed and were soxhleted using 300ml methanol. Soxhletion were continued till it undergo 20 cycles. The solvent was evaporated to make the concentrated extract and stored at 4°C (Harwood *et al*., 1999).

B..Antibacterial activity

Antibacterial activity was determined by disc diffusion method (Acar *et al*., 1991)

Sterile disc impregnated with the test substance were placed on the surface of the Muller-Hinton agar medium inoculated with the target organisms. From the stock solution of extract (0.1g/ml) 25µl, 30µl, 35µl, 40µl of the samples were tested for the activity. The bacterial cultures were adjusted to 0.5 McFarland turbidity standard and inoculated into Muller-Hinton agar, the cultures used are *Streptococcus pyogens* (*MTCC1928)*, *Staphylococcus aureus*(*MTTC 3160), Eschrichia coli* (*MTTC 40)*, *Salmonella typhi* (*MTCC 3224)* and *Klebsiella pneumonia (MTCC 7028).* Sterile filter paper discs impregnated with extract of different concentration were applied over each of the culture plates seeded with the 0.5 McFarland culture of bacteria. Distilled water and chloramphenicol was used as control. Bacterial cultures were then incubated at 35°C - 37°C for 24 hours. Antibacterial activity determined by measuring the zone of inhibition around each paper disc.

C.Minimum inhibitory concentration

Minimum inhibitory concentration (3-(4, 5-dimethylthiazol - 2 -yl 2, 5 -diphenyltetrazolium bromide) MTT (Sette *et al*., 2006, Buatong *et al*., 2011)

The minimum inhibitory concentration of the methanolic extract of fruit was performed by serial microplate dilution method of Eloff. The method was slightly modified by using 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) instead of p-iodonitrotetrazolium violet INT. This method allows the determination of the minimum inhibitory concentration (MIC) of extract against each bacterial species by measuring reduction of tetrazolium violet. The bacterial cultures were incubated in Mueller-Hinton (MH) broth overnight at 37 °C and a 1% dilution of each culture in fresh MH broth was prepared prior to use in the micro dilution assay. 100 µl of each bacterial culture and the extract were added to 96 well microtitre plates. The plates were incubated overnight at 37 °C and bacterial growth was detected by adding 40 µL MTT (5 mg/ml) (Sigma) to each well. After incubation at 37 °C for 1 h, MTT is reduced to a purple formazan by biologically active organisms. The well in which the solution remained clear was shown to inhibit the growth of bacteria. This concentration was taken to be the minimum

inhibitory concentration (MIC). Sterile broth containing extract alone and the standard antibiotic chloramphenicol (Sigma) were included in each experiment as negative and positive controls.

D.Preliminary phytochemical screening

The extract were subjected to various qualitative chemical tests for detecting the presence of phytoconstituents like alkaloids, flavanoids, tannins, saponins, phenolic compounds, carbohydrate, terpenoids, carotaenoid and phylobatannins. Screening of the extract for various phytochemical constituents was carried out using standard methods of Sofowora 1993.

E.Antioxidant activity

Determination of total antioxidant capacity by phosphomolybdenum method (Prieto *et al*; 1999)

The anti oxidant assay is based on the reduction of Mo(VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. An aliquot of 0.1ml sample was combined with 1.0ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against the blank. A typical blank contained 1.0 ml of reagent solution and the appropriate volume of the solvent used to dissolve the extract. For samples of unknown composition, antioxidant capacities were expressed as equivalent of ascorbic acid (µmol/g of sample).

F.Assesment of in-vitro ovicidal activity

(Jabbar *et al.,* 2007 and Rahman *et al.,* 2011).

Fresh ova were collected from faecal sample of goat infected with *Haemonchuscontortus* and were concentrated by centrifugation 2 min at 2,000 rpm, supernatant was poured off and the tubes were agitated to loosen the sediment and saturated sodium chloride solution. Eggs were washed by centrifugation with distilled water prior to the experiment. Albendazole were used as the positive control whereas distilled water served as the negative control. The extract was diluted to concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 mg/ ml. In this experiment, about 50 eggs/ 0.5 ml distilled water were counted and taken in marked 6 wells or tissue culture plates and were added with 0.5 ml of the extract as described earlier. The effective concentrations of the extract in each petriplate was thus reduced to 50, 25, 12.5, 6.25, 3.125 and 1.5625 mg/ml. Albendazole was also diluted using DMSO to provide concentration of 1 and 0.5 mg/ ml. The culture plates were incubated for 48 hours at 28°C. The experiment was done in triplicates for each concentration. Hatched larvae (dead/live) and unhatched eggs were counted under dissection microscope (magnification 40 X).

G.Assessment of cytotoxic activity

Assessment of Cytotoxic Activity using Brine Shrimp (*Artemia salina*) Lethality assay (Meyer *et al.,* 1993).

Brine shrimp lethality assay is a useful tool for preliminary assessment of cytotoxic and antitumor agents. It is based on the ability of the compound to kill laboratory cultured brine shrimp, *Artemia salina*. Sample was prepared by dissolving 100 mg of extract in 1ml of sterilized distilled water and serially diluted to 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 and 0.39 mg/ml. *Artemia salina* were hatched from its egg in natural sea water under constant aeration at room temperature.To 4.5 ml of filtered sea water taken in test tubes, 0.5 ml of the serially diluted samples was added and made up to 5 ml. The effective concentration of the extract in each tube thus gets reduced to 11.11, 5.55, 2.77, 1.38, 0.69, 0.34, 0.173, 0.086, and 0.043 mg/ml. Distilled water served as negative control. 20 active brine shrimps were added to each tube. It was incubated for 24 hours at room temperature and number of dead shrimps was counted. LC_{50} were determined using probit analysis.

III. RESULTS

A. Antibacterial activity

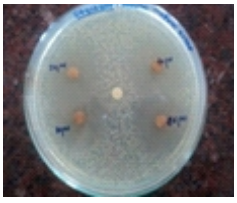
The antibacterial activity of methanolic extract of *Garcinia gummi-gutta*is shown in table no :1and Plate :1. The activity index was found to be maximum against *S.pyogenes* followed by *S.aureus*. The extract shows moderate level of activity against rest of the organisms.

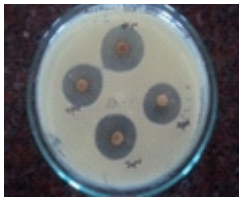
Table 1: Antibacterial Activity Of The Extract

Test microorganism	Inhibition zone of extract in mm Concentration (mg/ml)						Inhibition Zone of Chloramphenicol (2.5 mg/ml) mm
	4	3.5	3.0	2.5	2.0	1.5	
S.pyogenes (MTCC 1925)	28	23	18	15	10	-	25
S.aureus (MTCC 3160)	24	20	18	15	10	-	30
E. coli (MTCC 40)	20	17	14	10	-	-	30
K.pneumonia (MTCC 7028)	18	16	14	11	-	-	20
S.typhi (MTCC 3224)	18	15	13	10	-	-	33

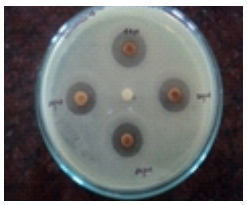
Plate:1Antimicrobial activity of *Garcinia gummi-gutta*

1.*S. pyogenes* 2.*S. aureus*

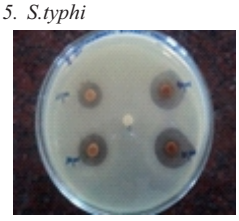




3.*Escherichia Coli* 4.*K.pneumonia*



5. *S.typhi*



B. Minimum inhibitory concentration

Minimum inhibitory concentration of the extract were obtained by using (3-(4, 5 -dimethylthiazol-2-yl-2, 5-diphenyltetrazolium bromide) MTT. The MIC results revealed that 2.0 mg/ml of the extract was enough for inhibiting *Streptococcus pyogenes* and *Staphylococcus aureus*, where as the MIC of *Klebsiella pneumoniae, Salmonella typhi*, and *Escherichia coli* were found to be 2.2 mg/ml shown in Table: 2.

Table 2: MIC of the extract

Organism	Minimum inhibitory concentration
Streptococcus pyogenes (MTTC 1928)	1.9 mg/ml
Staphylococcus aureus (MTCC 3160)	1.9 mg/ml
Salmonella typhi (MTCC 3224)	2.2 mg/ml
Escherichia coli (MTCC 40)	2.2 mg/ml
Klebsiella pneumonia (MTCC 7028)	2.2 mg/ml

C. Preliminary phytochemical screening

The qualitative analysis of phytochemicals in the methanolic extracts of *Garcinia gummi-gutta* indicated the presence phenols, phylobatannins, triterpenoids, saponins, steroids, reducing sugars and carbohydrates. The results are tabulated in Table: 3.

Table: 3 Phytochemical analysis of *G. gummi-gutta*

S.No	Phyto Chemical tests	Results
1.	Phenol	+
2.	Alkaloid	-
3.	Tanins	-
4.	Sapronin	+
5.	Terpenoid	+

6.	Steroid	+
7.	Carbohydrate	+
8.	Reducing sugar	+
9.	Phylobatannin	+

D.Antioxidant property

Antioxidant property of extract was found by using phosphomolybdenum method, by comparning with ascorbic acid by taking the same concentration of both extract and ascorbic acid (25 µg/ml – 125 µg/ml).(Table: 4)

Table 4: Antioxidant property of the extract

S.NO	Conc µg/ml	Volume (µl)	Optical density	
			Ascorbic acid	Extract (test)
1.	25	1	0.20	0.50
2.	50	2	0.36	0.12
3.	75	3	0.52	0.26
4.	100	4	0.69	0.40
5.	125	5	0.85	0.55

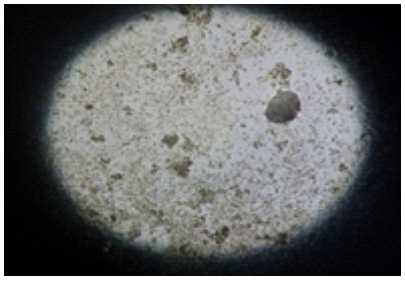
E. Assesment Of In-Vitro Ovicidal Activity

The extract showed inhibition of hatching of eggs in a dose dependant manner. Albendazole at both dose produced 100% of death. There was destruction of the entire number of ova counted. The results are tabulated. (Table: 5)

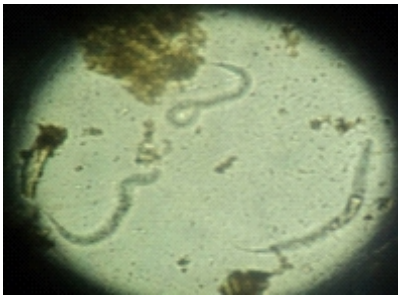
Table 5: *Invitro* ovicidal activity of the extract

S. No	Concentration Of extraction (mg/ml)	No.of eggs	Observation dead/hatched
1	50	50	Dead LI stage
2	25	50	Dead L1 stage
3	12.5	50	Dead L1 stage
4	6.25	50	Dead L1 stage
5	3.125	50	Dead L1 stage
6	1.5625	50	Dead L2 stage

Plate No :2 Ovicidal activity of extract at 50mg/ml



Negative control



IV. DISCUSSION

The phytochemical screening of the fruit extort revealed that the material contains significant amount of phenols, saponins, phylobatannins, terpenoids, steroids, carbohydrates, reducing sugar,. Phytochemical investigation conducted on the fruit extract disclosed medicinal as well as physiological activites (Tarali chowdhury, 2014).

Phenols are found in the natural world, above all in the plant kingdom. The antioxidant activity of phenol is mainly due to their redox properties, hydrogen donor and singlet oxygen quenchers. A few phenols are proved to have hypotensive and antioxidant properties. Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al*., 2007). Naturally antioxidant in plants is in the form of phenolic compound . *G. gummi-gutta* contains many antibacterial substance which could have contributed for the high-quality activity against microbes. Further investigation is essential to isolate and identify the compound for therapeutic purpose.

The presence of saponins also shows the potential of the plants to be used to produce mild detergents and intracellular histochemistry staining to allow antibody access to intercellular proteins . This can be attributed to the ability of saponins to bind with glucose and cholesterol molecules. Saponins have also been connected with inhibitory effect on irritation (Just *et al*., 1998). Terpenoids have medicinal value such as anti-carcinogenic, antimalarial, antimicrobial and diuretics activity (Deganhardt, 2003). Terpenoids have also shown a great potential in treatment against disease causing microorganisms.

The extract also contain plenty amount of phylobatannins and steroids .Which were found to be present in all the extract of the plant parts and they are of tremendous significance and interest in pharmaceutical research.(Rao *et al*., 2003, Okwu & Okwu., 2004).

It also contains carbohydrates. The carbohydrates in food are of major interest in relation to chronic diseases. Different types of carbohydrates give to different glycemic responses, and also able to inspire lipogensis . The result aquired in this study thus suggests that the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be a valuable reservoir of bioactive compounds of substantial medicinal merit.

Phenolic compound of plant acts as primary antioxidants or free radical scavenger (Ayoola *et al*., 2008). Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals (Shahidi *et al*., 1992). It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health beneficial effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores (Vaya *et al*., 1997). The phenols contain hydroxyls that are responsible for the radical scavenging effects mainly due to the redox properties . According to recent reports, a highly positive relationship between total phenol and antioxidant activity appears to be the trend in many plant species.

The reducing power activity of the compounds could serve as a significant indicator of the antioxidant potential, in this study this were assessed this property by measuring the ability of the extract to transform Fe3+ to Fe2+ and to donate an electron(Rao *et al* ., 2010). The ability of the extracts to reduce Fe3+ could be attributed either to the reducing agents such as phenol groups and the number or/and the position of the hydroxyl molecule on these groups.

The natural compounds are usually occurring with some non-enzymatic compounds such as ascorbic acid, phenolic compounds, flavanoids etc; therefore the discovery of natural antioxidant is a major research area now a day. In my study the antioxidant property of the extract were assessed by using phoshomolybdenum method .

Brine shrimp nauplii have been used previously in a number of bioassay systems. The brine shrimp assay has advantage of being rapid (24hrs), in expensive and simple. It easily utilizes a large number of organisms for statistical validation and requires no special equipment and a relatively small amount of simple. It is a well-situated preliminary toxicity test since brine shrimp are highly sensitive to a variety of chemical substance (Franssen *et al*., 1997).the result of the brine shrimp lethality bioassay indicates the presence of active cytotoxic component present in the extract.

The antibacterial effect was determined using the disk diffusion method as outlined in. The zones of bacterial inhibition were measured to the nearest whole millimeter (mm). Diameter of zone of inhibition >10 mm were considered active . The antibacterial activity found in the plant extracts have been attributed to some of the secondary metabolites (Cowman, 1999). The MIC of the given sample was estimated by using MTT (3-4, 5-dimethylthizol -2-yl-2, 5-

diphenyltetrazolium bromide (Sette et al., 2006 Buatong *et al*., 2011).

In the search for natural anthelmintics, *in vitro* tests are used as preliminary studies of plants. In these tests, the plant extracts are directly placed in contact with the eggs, larvae or adult parasites to evaluate the effect on egg hatching, larval development or motility and mortality of adult worms (Hammond, 1997). The anthelmintic activity of the extract may be attributed to presence of phytochemicals, like Saponins destabilize membranes and increase cell permeability by combining with membrane-associated sterols (Gee *et al*.; 1988). The use of botanical anthelmintics has been proposed as an alternative strategy for the control of gastrointestinal nematode infections in order to reduce the belief on chemical anthelmintic treatments and to setback the selection and the transmission of anthelmintic resistances in worm populations (Hoste *et al*., 2006).

V. Conclusion

The enduring medication of antibiotic, over treatment of synthetic antioxidants and chemical anthelmintic treatment has a possibility for causing wellbeing hazards and side effects . The chief aim of this study was to find a natural therapeutic compound which can be an substitute in treating with drug. The present study throws light into the depth of phytochemicals which posses' unique characteristics. In futher study these phytochemicals and bio active molecules should be quantified and detailed studies have to be conducted to reveal the structure of the compounds. The bio active molecule should be developed and can be reached in market level. Hence this study was conducted to reveal the importance of biomolecules from *Garciniagummi-gutta*..

Reference:

[1] Ayoola .G.A.,Coker,H.B.,Adesegun,A.A.,Bello,K.,Obeweya E.C."Phytochemical Screening of some selected medicinal plant used for Malaria therapy in south western Nilgires. J Pharm. Res., 7pp.1019-102.2008.

[2] DmitriyObolskiy,Iyo, P.and Micheal, .Phytotherapy a research. Vol 23, 1047-1065.2009.

[3] Jabbar A, Zamana MA, Iqbala, Z. Yaseenb, M., Shamima A. "Antihelmintic activity of Chenopodiumalbum(L.) and Caesalpinia crista (L.) against trichostrongylid nematodes of sheep", J Ethanopharmacol;144(1):86–91. 2007.

[4] Just M.A., Carpenter P.A., Keller TA, Eddy WF, Thulborn KR. "Brain activation modulated by sentence comprehension". Science; 274: 114–16. 1996.

[5] Rahman," Effect of delonixregia leaf extract on glucose tolerance in glucose". Afr J Tradit Complement Altern Med. 8(1):34-36. 2011.

[6] TaraliChowdhury , Virtual screening of compounds derived from Garciniapedunculata as an inhibitor of gamma hemolysin component A of Staphylococcus aureusBangladesh J Pharmacol 9: 6771 . 2014.

[7] Acar .J.F and Goldstein F.W." Disk susceptibility test . In: antibiotic in laboratory medicine", Loran (Ed),3rdEdn.1991.

[8] Brantner A, Males Z, Pepeljnjak S, Antolic A. Antimicrobial activity of Paliurusspina-christi Mill (Christ's thorn). J. Ethnopharmacol. 52: 119-122. 1996.

[9] Buatong J, phongpaichits,Rukachaisirikuiv, Sakayaroj J. antimicrobial activity of crude extracts from mangrove fungal endophytes .World J microbial Biotechnol27(12):3005-3008. 2011.

[10] Deganhardt, J. Attracting friends to feast on foes: Engineering terpene emission to make crop plant more attractive to herbivore enemies. Curr.Opin.Biotechnol ,14:169-176. 2003.

[11] Harwood. L.M, Moody C..J., Percy J.M . Experimental Organic Chemistry 2nd. Blackwell Science Ltd, Oxford, UK,. 1989.

[12] Okwu, D. E. &Okwu, M. E. "Chemical composition of Spondiasmombinlinn. plant part", J. Sustain. Agric. Environ. 6(2), pp. 140-147. 2004.

[13] Rao, Ch. V., OjhaAmresh, S. K., Mehrotra, S. &Puspangadan, P. "Analgesic, Antiinflammatory and Antiulcerogenic Activity of Unripe fruits of Aeglemarmelos", ActaPharmaceuticaTurcica 45, pp. 85-91. 2003.

[14] Shahidi, F., Janitha, P.K. and P. Wanasundara. Phenolic antioxidants. Critical Reviews in Food Science and Nutrition 32:67-102. 1992.

[15] Vaya J., Belinky P. A and Aviram M. Antioxidant constituents from Licorice root: Isolatoin, sture elucidation and antioxidative capacity towards LDL oxidation, Free radical Bio. Med. 23(2): 302-312. 1997 .

[16] Hammond J.A., Fielding D., Bishop S.C. 1997. Prospects for plant anthelmintics in tropical veterinary medicine. Vet Res Commun. ;21:213–228.

[17] Gee J. M., Blackburn N. A., Johnson I. T. The influence of guar gum on intestinal cholesterol transport in the rat. Br. J. Nutr. 50:215–224.1983.

[18] Hoste H.,Jackson F.,Athanasiadou S.,Thamsborg S.M.and Hoskin S.O. The effects of tannin rich plant on parasitic nematodes in ruminants. Trends Parasitol.22.p.253-261.2006.

[19] Sette.L.D, Passanini M.R.Z., Delarmelia ,C., Salafi.F., Duart, M.C.T., molecular characterization and antibacterial activity of endophytes. World J Microbial Biotechnol 27(12):3005-3008.2011.

[20] Sofowora A (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp. 191-289.

[21] Prieto, P., Pineda, M., Aguilar, M: Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry 1999; 269:337–341.

[22] Meyer, B. N., Ferrighi, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. & McLaughlin, J. L. (1982). Brine shrimp: A convenient general bioassay for active plant constituents. Planta Medica, 45, 31-34.

[23] Singh, R. P. ; Dhania, G. ; Sharma, A. ; Jaiwal, P. K., 2007. Biotechnological approaches to improve phytoremediation efficiency for environment contaminants. In: Environmental bioremediation technologies, Springer, 223-258.

[24] Franssen, F F, Smeijsters F.and Medinilla Aldana B.E.In vivo and in vitro antiplasmodial activities of some plants traditionally used in Guatemala against malaria.Antimicrob. Agents Chemother. July 1997 ; 41:7 1500-3