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HERBAL FORMULATIONS OF *VAIVILANGAM CHOORNAM*: AN APPROACH FOR THE TREATMENT OF ANTHELMINTIC IN INDIAN CHILDREN

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ABSTRACT

Worms infect more than one third of the world's population, with the most intense infections in children and the poor. About half of the population in South India and 50% of school children in tribal areas of Central India are infected with *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms. In Siddha system, many herbs and formulations are indicated and practiced for Anthelmintic action. The two herbs chosen for the current study *Kattuseeragam* and *Vaivilangam* have been mentioned in the Siddha literature as having anthelmintic properties. However there are no proved scientific supporting preclinical data yet now in the combination therapy of kattuseeragam and vaivilangam. Various research studies on traditional medicines are needed to standardize the Siddha medicine similar to Modern Medicine. However, its prospective will depend on proving its significance through scientific tests of verifiability and reproducibility. The current study was aimed to formulate *vaivilangam choornam* for the treatment of intestinal helminthes caused by heavy worm burden in young children. From the various pre-clinical studies like phytochemical studies, microbial content assay, heavy metal analysis, aflatoxins, pesticides residuals and *in vitro* anthelmintic activity of *vaivilangam choornam* compared with modern medicine tablet albendazole proves that *vaivilangam choornam* will be the right choice and safe medicine for the treatment of intestinal helminthes infestations in Indian children.

KEYWORDS

Vaivilangam choornam, Anthelmintic and Indian children.

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INTRODUCTION

Siddha medicine is an Indian traditional medicine practice system, continued from our ancient era, to treat and heal the common people of India. In earlier days of practice literature passed through oral lineage from the master to the disciple and later through palm leaves manuscripts. Nevertheless, in modern era, the enormous literature of siddha medicine is being kept still active and considerable

to the health needs of the modern community, through printed literature and electronic media. In the current study, is on the way of focus to prove the significance of using combination of kattuseeragam and *vaivilangam choornam* for the treatment of intestinal helminthes caused by heavy worm burden in young children. Many research studies have reveals that among 50% of central tribal part of young children have a high infection rate and suffer with intense worm burden of *A. lumbricoides*, *Trichuris trichiura* and hookworms. These parasitic infections are noticeable themselves resulting in reduced growth rates through impaired nutrient utilization. Moreover, heavy hookworm burden is the major etiology for iron deficiency anemia in young children. In Siddha medicine system, many herbs are in the practice for treating helmenthic diseases, among that kattuseeragam and vaivilangam are widely used. However there are no proved scientific supporting preclinical data yet now in the combination therapy of kattuseeragam and vaivilangam. This study is considered to generate supportive scientific data using current preclinical research on the combintion of herb kattuseeragam and vaivilangam. The present study deals the Identification and authentication of ingredients of the formulation, Physio-chemical, phytoconstituents evaluation of the formulation test for heavy metals, microbial contamination, specific pathogens, pesticide residue and aflatoxin, finger printing of the formulation by TLC methods and *in vitro* antihelminth activity of the formulation. vaivilangam is comprised of embelic acid, colouring matter, tannin, a resinoid body and alkaloid called cbristembeine (check spelling). Fruit, Seed of vaivilangam have been used for the treatment of Aneamia, abdominal disorders. Obesity, Snake bite and rat bite poisonings, worm infestation, digestive disorders and skin diseases. Seeds of kattucheeragam contain resins, an alkaloid known as vernonine, tannins, glycosides, saponins, phlobaphenes and steroids. Powdered seeds in doses of 30-60 grains have a weak vermifugal action against ascariasis and more powerful action against oxyuris[1-10].

MATERIAL AND METHODS

Preparation of *vaivilanga Chooranam*

The raw drug of Kattuseeragam and vaivilangam were purchased from a well reputed country shop and raw drugs were authenticated by the help of Head of Department of Medicinal Botany, National Institute of Siddha, Chennai, and Tamilnadu. The medicine was prepared as per Good Manufacturing Practice (GMP) guidelines of the Drugs and Cosmetics act 1947 in Drug Standardization Laboratory (DSL), at Department of Siddha, The Tamilnadu Dr MGR Medical University, Chennai, Tamilnadu[11-15].

Intially, the seeds of kattuseeragam and vaivilangam has cleaned and purified. After purification of the seeds, the seeds were powdered separately and mixed well in equal part. Milk was taken in the mud pot and covered with plain cloth. Mixed drug was placed in the cloth and covered with lid. Above prepared pot was placed on the stove and boiled till the powder was cooked. Then the powder was dried in shadow and stored in air tight container (Figure No.1).

Physicochemical Evaluation[16-35]

Organoleptic evaluation

Vaivilangam Chooranam was subjected for organoleptic evaluation such as size, colour, odour and taste. The herbs were collected from the botanist in the National Institute of Siddha, Chennai.

Determination of moisture content (loss on drying)

Determination of moisture content is done by the standard protocols.

Determination of total ash value

2gms of powdered crude drug were weighed accurately in previously ignited and tarred silica crucible and gradually increasing the temperature to 400^{o}C until it appeared white ash material indicates the absence of carbon. Total ash in mg per gm is calculated.

Determination of Acid insoluble ash

Boil the total ash with for five minutes with 25 ml of dilute hydrochloric acid, collect the insoluble matter in a Gooch crucible or on an ash less filter

paper, wash with hot water, ignite, and weigh. Calculate the percentage of acid- insoluble ash with reference to the air dried drug.

Determination of water soluble ash

The total ash was boiled for 5 minutes with 25ml of water; insoluble matter was collected on ash less filter paper wet with hot water ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the ash. The difference in the weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to air dried drug.

Determination of Extractive values

5gms of dried coarse powdered drug was accurately weighed and placed inside a glass stoppered conical flask. It is then macerated with 100ml of chloroform water for 18 hours. It was then filtered and about 25ml of filtered was transferred into a china dish and was evaporated to dryness on a water bath. It was then dried at 105°c for 6 hours, cooled and finally weighed.

Alcohol soluble extractive

5gms of dried coarse powdered drug was accurately weighed and placed inside a glass stoppered conical flask. Above mixture was macerated with 100ml of ethanol for 18 hours. Filtration was processed after maceration and about 25ml of filtrate was transferred into a china dish and evaporated on a water bath. It was then dried at 105°c for 6 hours, cooled and finally weighed.

Phytochemical Analysis

The aqueous methanol extract of *vaivilangam chooranam* was freshly prepared and divided into different test tubes and various chemical constituents (tannins, saponins, glycosides, alkaloids, triterpenes, polyphenol and flavanoids) were analysed according to standard methods[41].

Test for Steroids (Salkowski Test)

Vaivilangam chooranam aqueous methanol extract was added to 2 ml of chloroform solution. A reddish brown color formation after addition of sulphuric acid indicates the presence of steroids.

Test for anthraquinone (Borntrager's test)

In a dry test tube 5 ml of *vaivilangam chooranam* aqueous methanol extract is added with 5 ml of chloroform and shaken for 5 min. The extract was filtered and the filtrate is added with equal volume of 10% ammonia solution and mixed. A pink violet or red color in the ammoniacal layer (lower part of tube) indicates the presence of anthraquinone.

Test for reducing sugar

5 ml of *vaivilangam chooranam* aqueous methanol extract was boiled on a water bath. After that to the above 5 ml of solution, 1 ml each of Fehling's solution A and B were added. The mixture was shaken and heated in a water bath for 10 min. A brick-red precipitate indicates the presence of reducing sugar.

Test for alkaloids

5 ml aqueous methanol extract of *vaivilangam chooranam* was diluted to 10 ml of acid alcohol, boiled and filtered. To 5 ml of above filtrate was added to 2 ml of dilute ammonia, continued with addition of 5 ml of chloroform and shaken gently to extract the alkaloid base. The chloroform layer was extracted with 10 ml of acetic acid. Then this extract was divided into two portions. To the one portion of extract is treated with Mayer's reagent and the other portion with Dragendorff's reagent. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was shows as positive report for the presence of alkaloids.

Test for flavonoids

Three distinct methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. During stand by condition, disappearance of yellow color indicates the presence of flavonoids. In the second method, a few drops of 1% aluminum solution were added to a portion of the filtrate, formation of yellow color indicates the presence of flavonoids. Third method, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was mixed with 1 ml of dilute ammonia solution. A yellow color formation indicates the presence of flavonoids.

Test for terpenoids (Nollers-test)

2 ml of aqueous methanol extract of *vaivilangam chooranam* was added to 2 ml of chloroform and concentrated H_2S0_4 respectively, was added carefully to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for saponins

3 ml aqueous methanol extract of *vaivilangam chooranam* was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins

5 ml of aqueous methanol extract of *vaivilangam chooranam* was boiled in water bath and then filtered. To the solution added few drops of 0.1% ferric chloride and observed for brownish green or a blue-black coloration which indicates the presence of tannins.

Test for cardiac glycosides (Keller-Killiani test)

5 ml of aqueous methanol extract of *vaivilangam chooranam* was added with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for phenols

5ml of filtered extracts were taken and 1ml of $FeCl_3$ (1%) and 1ml K_3 (Fe (CN) $_6$) (1%) were added. The appearance of reddish blue color indicated the presence of polyphenols.

Anthelmintic Assay

The assay was performed on adult Indian earthworm *Pheretima posthuma* due to the anatomical and physiological resemblance with the intestinal roundworm parasite of human beings and also easily available earthworms have been used widely for the initial evaluation of anthelmintic activity. The *in vitro* anthelmintic activity was determined by releasing into 10 ml of desired formulation containing three different concentrations, each of *vaivilangam chooranam* (20, 40 and 60mg/ml in distilled water) were prepared. Albendazole (10mg/ml) was used as reference standard while distilled water used as control and six worms (same type) were placed in petri dish. Observations were made till time taken to paralysis and/or death of individual worms. Paralysis is said to occur when the worms do not revive even in normal saline water. Death was concluded when the worms lose their motility followed with fading away of their body colour or not moved when dipped in warm water at 50ºC[1-3].

RESULTS[36-51]

The botanical identification and authentication were done for the ingredients seeds of kattujeeragam and seeds of vaivilangam. Organoleptic identification of *vaivilangam chooranam* was done by their color, appearance, taste and surface characters as described in the Siddha pharmacopeal standards. The physiochemical parameters like loss on drying, total ash, acid insoluble ash, water soluble extractive and alcohol soluble extractive were analyzed for the *Vaivilangam chooranam* and reported in Table No.1.

The phytochemical screening report of the *Vaivilangam chooranam* revealed the presence of alkaloids, flavonoids, tannin and reducing sugar (Table No.2).

***In vitro* Anti-anthelmentic Activity**

The *in vitro* anti anthelmentic activity results indicate that significant dose dependent anti-anthelmentic effect of the siddha formulation against adult Indian earthworm *Pheretima posthuma*. The higher dose of the formulation has better anti anthelmentic activity in comparison to standard drug albendazole (60mg/kg). The siddha formulation (60mg/kg) has taken more time to induce paralysis and death in the worm. The results are showed in the Table No.3.

DISCUSSION

Pre-clinical studies

In the present study, the moisture content of the *Vaivilangam chooranam* was found to be 17%. The acid insoluble ash value and water soluble ash values were 2.5% and 3.5% respectively and the total ash was 5.5% which are within the permissible limits. Pre-clinical studies data reveals that the study drug is suitable to be used as internal medicine.

A preliminary phytochemical study shows that rich availability of major phytochemicals in the formulation through the confirmation test on study drug; shows the presence of reducing sugar, tannins, saponin, glycosides, alkaloids, triterpenes, polyphenol and flavonoids in the study drug.

Tannins are known to produce anthelmintic activity by binding to glycoproteins on the cuticle of the parasite. The hinder energy production in the helminthes parasite is by interfering with oxidative phosphorylation.

In vitro anthelmintic activity of *vaivilangam choornam* was evaluated by using adult Indian earth worm (*Pheretima posthuma*) and the result data were compared with modern formulation of albendazole tablet (Figure No.2).

Table No.1: Physicochemical parameters of the *vaivilangam chooranam*

S.No	Test	Result
1	Description Colour Odour Taste	Reddish Brown Woody odour Slightly bitter in taste
2	Loss on drying at 105^0	2.2%
3	Total -ash	5.5%
4	Acid -Insoluble ash	1.32%
5	pH	6.6
6	Total solid	97.8%
7	Fat content	Absent
8	Reducing sugar	Absent
9	Total sugar	Absent

Table No.2: Phytochemical screening of extract of *Vaivilangam chooranam*

S.No	Phytochemical Constituents	Observation	Extract of drug
1	**Alkaloids** Mayers test Dragendorff's test	Yellowish precipitation Orange / red precipitate	+ +
2	**Flavonoids** Alkali Reagent Lead acetate test	Intense yellow colour Yellow precipitate	+ +
3	**Glycosides** Keller-Killiani test Borntrager's test	Reddish brown color ring Pink colour (Ammonia layers)	+ -
4	**Tannins** $FeCl_3$ test	Blue black colour	+
5	**Saponins** Frothing test	Foam	+
6	**Terpenoids** Salkowski test	Reddish brown ring formed in interface	+
7	**Polyphenols** Ferrozine test	Reddish blue color	+
8	**Anthocyanines** Ammonia test	Yellow color	+

- Negative (absent) + Positive (present)

Table No.3: Anthelmintic Activity of *Vaivilangam chooranam*

S.No	Treatment	Conc. Used	Time taken (min)	
1			**For paralysis**	**For death**
2	Vehicle (Distilled Water)	10ml	10ml	10ml
3	Albendozle	60mg	4.24 ±0. 20	9.20 ± 0.16
4	Vaivilangam chooranam	20mg	42±0.14	90±0.54
5	Vaivilangam chooranam	40mg	36±0.22	78±0.56
6	Vaivilangam chooranam	60mg	27±0.42	62±0.26

One way ANOVA followed by Dunnet's test. Values are mean ± S.E.M. n = 6 in each group.

Figure No.1: Final formulation of *vaivilangam choornam*

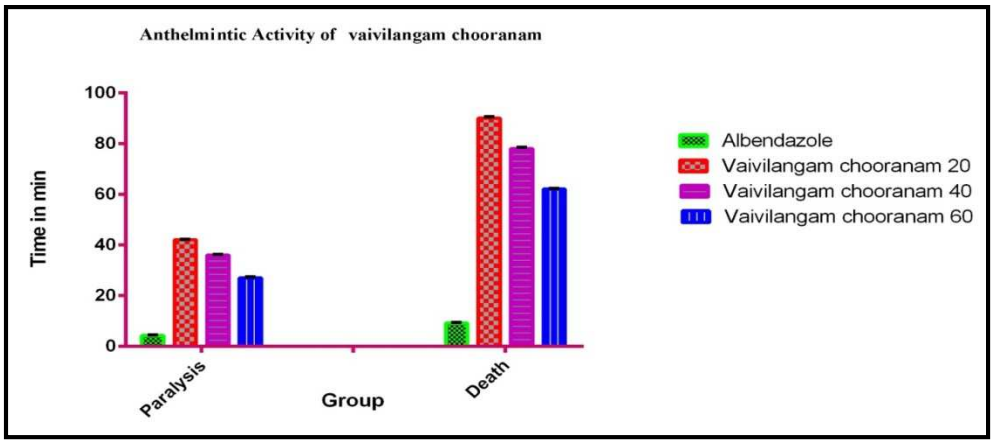

Figure No.1: Anthelmintic Activity of *Vaivilangam chooranam*

CONCLUSION

Preclinical studies report reveals that siddha herbal formulation of *Vaivilangam chooranam* are suitable internal drug for the anthelmintic treatment in human. Phytochemical study report proves that the presence of tannins and tannins are known to produce anthelmintic activity by binding to glycoprotein on the cuticle of the parasite. The hinder energy production in the helminthic parasite is by interfering with oxidative phosphorylation. Comparative in vitro anthelmintic activity studies on *vaivilangam choornam* and albendazole data suggests that, *Vaivilangam choornam* will be a promising herbal formulation for the treatment of anthelmintics in children.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Lorenzo Savioli. *Indian J Med Sci*, 62(12), 2008, 485-493.
2. Chakma T. Prevalence of anaemia and worm infestation in tribal areas of Madhya Pradesh, *J Indian Med Assoc*, 98(9), 2000, 567-71.
3. Albonico M, Allen H, Chitsulo L, Engels D, Gabrielli A F, Savioli L. Controlling soil-transmitted, *Ind J Med Sci*, 62(4), 2008, 484-491.
4. Awasthi S, Bundy D A P, Savioli L. Helminthic Infections, *BMJ*, 19, 2003, 885-888.
5. Kvalsvig J D, Cooppan R M, Connolly K J. The effects of parasite infections on cognitive processes in children, *Ann Trop Med Parasitol*, 85(5), 1991, 551-68.
6. Roche M, Layrisse M. Hookworm anaemia, *Am J Trop Med Hyg*, 15(6), 1966, 1029-102.
7. Guyatt H. Do intestinal nematodes affect productivity in adulthood? *Parasitol Today*, 16(4), 2000, 153-8.
8. Crompton D W, Nesheim M C. Nutritional impact of intestinal helminthias is during the human life cycle, *Ann Rev Nutr*, 22(1), 2002, 35-59.
9. Chan M S, Medley G, Jamison D, Bundy D A. The evaluation of potential global morbidity attributable to intestinal nematode infections, *Parasitology*, 109(Pt3), 1994, 373-87.
10. Murukesa mudaliyar K S. *Siddha Materia Medica, Chennai, Department of Indian Medicne and Homoeopathy*, 5th Edition, 1998, 535-536.
11. *Pakistan Journal of Pharmaceutical Sciences*, 3(2), 1990, 69-74.
12. Surya Hadi and John B B. Initial Studies on Alkaloids from Lombok Medicinal Plants,

Molecules, 6(2), 2001, 117-129.
13. Lohar D R. Protocol for testing of Ayurveda, Siddha, Unani Medicines, *Ghaziabad, Pharmacopoeial Laboratory for Indian Medicines, Department of AYUSH*, 2007.
14. WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems, *World Health Organization, Geneva*, 2004, (http: // apps.who.int/ medicinedocs/ documents/ s7148e/ s7148e.pdf).
15. Sambasivam Pillai T V. Siddha Medical dictionary, Chennai, *Department of Indian Medicne and Homoeopathy*, 2nd edition, 2(2), 2006, 278.
16. Madhalai noi.
17. Sundararasan A. Pillaipini maruthuvam, Chennai, *Department of Indian Medicne and Homoeopathy*, 1st edition, 1993, 995-997.
18. Jeeva ratchamirtham.
19. Murukesamudaliyar K S. Kuzhanthai Maruthuvam (Balavagadam), Chennai, *Department of Indian Medicne and Homoeopathy*, 4th edition, 2007, 1031-1040.
20. Ramachandran S P. Dhanvandhri Vaidhyam - 1000, Chennai, *Tamarai Noolagam*, 1st edition, 1997.
21. Kumbamuni Balavagadam.
22. Sivaprakasam V. Pararasasekaram Balaroganithanam, Chennai, *Tamarai Noolagam*, 1st edition, 2003, 585-592.
23. Sowrirasan M. Sarabendhira siddha maruthuva sudar, Chennai, *Tamarai Noolagam*, 5th edition, 2007, 890-925.
24. Gurunadi.
25. Rathina Surukkanadi.
26. Uttamarayan K S. Siddhamaruthuvaanga surukkam, Chennai, *Department of Indian Medicne and Homoeopathy*, 2nd revised edition, 2006.
27. Uttamarayan K S. Thotrakiramaaratchi, Chennai, *Department of Indian Medicne and Homoeopathy*, 4th revised edition, 2008.
28. Subhash Chandra Parija. Medical Parasitology, *All India Publishers and Distributors, New Delhi*, 4th edition, 3(1), 2013, 93-94.
29. Jayaram Paniker C K, Sougata Ghosh. Medical Parasitology, *Jaypee Brothers Medical Publishers (p) Ltd, New Delhi*, 7th edition, 2013, 266-272.
30. Gasser, Cantacessi, Campbell. Improved molecular diagnostic tools for human hookworms, *Expert Rev Mol Diagn*, 9(1), 2009, 17-21.
31. Hotez P, Bethony J, Bottazzi M E, Brooker S. Buss P. Hookworm: “The Great Infection of Mankind”, *PLoS Med*, 2(3), 2005, e67, 177-181.
32. John, David T and William A, Petri, Markell J R and Voge’s. Medical Parasitology, *St. Louis: Saunders Elsevier*, 9th edition, 2006, 1073-1077.
33. Hotez P, Bethony J, Bottazzi M E, Brooker S, Buss P. Hookworm: “The Great Infection of Mankind”, *PLoS Med*, 2(3), 2005, e67, 177-181.
34. Charrerjee K D. Parasitology, *CBS Publishers and Distributors Pvt Ltd, New Delhi*, 13th edition, 2009, 159-166.
35. Nadkarani K M. Indian Materia Medica, *Popular Prakashan Pvt. Ltd, Bombay*, 3rd revised and enlarged edition, 2007, 357-358.
36. Kirtikar K R and Basu B D. Indian Medicinal plants, *International Book Distributors, Dehradun*, 2nd edition, 2006, 149-162.
37. Paranjpe A S and Gokhale G K. *Arch .int.pharm.et Ther*, 1932, 42-11.
38. Wealth of India.
39. Pharmacopoeia Indica, 84.
40. Trease G E, Evans W C. Pharmacognsy, *Brailliar Tiridel Can, Macmillian publishers*, 11th edition, 1989, 568.
41. Harborne J B. Phytochemical methods, *Chapman and Hall, Ltd, London*, 1st edition, 1973, 49-188.
42. Bate-Smith E C. Colour reaction of flower attributed to a (a) flavanols and (b)

carotenoid oxides, *J. Exper. Biol*, 64(1), 1953, 1-7.
43. Kovalev S V. Flavonoids from Lotus ucrainicus and I arvensis, *Chemistry of Natural Compounds*, 45(4), 2009, 550-551.
44. Limmatvapirat C J, Charoenteeraboon, Phaechamud T. Simultaneous analysis of eleven heavy metals in extracts of Sonneratia caseolaris (L.) *Engl. By ICP-MS. Res. J. Pharm, Biol. Chem. Sci*, 3(1), 2012, 744-50.
45. Magwood S E. Evaluation of a Method for Determining Microbial Counts of Hatcher Fluff, *Avian Diseases*, 6(1), 1962, 24-32.
46. Luciana de CASTRO. Determining Aflatoxins B1, B2, G1 and G2 in Maize Using Florisil Clean Up With Thin Layer Chromatography and Visual and Densitometric Quantification, *Ciênc. Tecnol. Aliment*, 21(1), 2001, 115-122.
47. OECD. OECD series on testing and assessment, guidance document on acute oral toxicity testing N 420: *Acute Oral Toxicity - Fixed Dose Procedure*, 2001.
48. OECD. OECD guidelines for the testing of chemicals
    N 407: *Repeated Dose 28 - Day Oral Toxicity Study in Rodents*, 2008.
49. Ajaiyeoba E O, Onocha P A and Olarenwaju O T. *In vitro* anthelmintic properties of Buchholzia coriaceae and Gynandropsis gynandra extracts, *Pharmaceutical Biology*, 39(3), 2001, 217-220.
50. Tambe V D, Nirmal S A, Jadhav R S, Ghogare P B, Bhalke R D, Girme A S, Bhamber R S. Anthelmintic activity of Wedelia trilobata leaves, *Ind. J. Nat. Prod*, 22(3), 2005, 27-29.
51. Mali R G, Mahajan S G, Mehta A A. *In-vitro* anthelmintic activity of stem bark of Mimusops elengi Linn, *PHCOG MAG*, 3(10), 2007, 73-76.

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