Assessment of Anthelmintic Activity of *Pisum sativum*

Omkar A.Devade*, Sachin A.Mehta1,3, Shashikant B. Lohare5, Ruchita S Takawale1, Dhanashree Y. Jadhav4

*Assistant Professor, Department of Pharmacology, AISSMS College of Pharmacy, Pune-India.
1,2,3,4 AISSMS College of Pharmacy, Pune-India.

*Corresponding author’s E-mail: om.devade@gmail.com

Received: 16-06-2022; Revised: 24-08-2022; Accepted: 29-08-2022; Published on: 15-09-2022.

ABSTRACT

In Indian medicinal plant literature, plant of *Pisum sativum* have been traditionally reported medicinal value as astringent, anticancer activity, Diuretic activity, Hepatoprotective activity, Antispermatogenic activity, Antioxidant activity, Antidiabetic activity. The extract of Whole plant of *Pisum sativum* was screened for anthelmintic activity on Indian earth worm in comparison to standard drug Albendazole. The concentrations *Pisum sativum* extracts and Albendazole were kept same for comparative activity. Saline water was kept as control. Determination of anthelmintic activity was done by recording the paralysis time and death time. Phytochemical test on plant extracts were carried out. The result showed that the anthelmintic activity of plant extracts was comparable to that of the reference drug Albendazole.

Keywords: *Pisum sativum*, Helminthiasis, Anthelmintic activity, Albendazole.

INTRODUCTION

*H*elmints is a Greek word meaning “worm”. It was supposed to be used only for intestinal worms but now includes tissue parasite free living species and mainly other worms1. Helminth infection are common parasitic infection affecting large number of populations, especially children. Helminths could be classified into three classes as Nematodea, Cestoidea and Trematodea. A wide range of worms are observed varying size from less than 1 mm – 1 m. People living in Australia, south-east Asia, India, Mexico, Sri Lanka, and Thailand are most affected by this infection. African people living in Sub-Saharan region are one of the most affected peoples2. From the current assessment 12-13% of world population have been infected by helminths which causes to severe morbidity it occurs due to persistence shortage, reduction in efficiency and poor socio-economic growth. AIDS, Malaria and Tuberculosis are more prone to be infected by helminthic. Inappropriate Sanitization is the primary reason of the Helminths infections in peoples. They mainly enter through contaminated drinking water or raw meats from infected animals. They may also get enter in body through skin by insect bites or walking and swimming in contaminated soil and water3. Helmets are host dependent macro-parasite. They require living host for survival, reproduction causing physical, nutritional, cognitive impairment in young children4. The life cycle of Helminths is complicated which requires several hosts. The primary host for helminths infection is humans, in which worms reproduce sexually which leads to form eggs or larvae which could affect secondary host.

Common symptoms of helminths infections are:
- Abdominal pain
- Hypoproteinemia
- Nausea
- Diarrhea
- Cough
- Slow down the mental growth and physical development

Anthelmintic drugs are drugs which are used for treatment of helminthic infections caused by various worms. These are the drugs which act locally to emit the worms from GIT or may also act systematically to eliminate adult helminths and prevent tissue and organs from developmental forms5. Anthelmintic drugs usually kill worms by either starving them to death or paralyzing them as worms don’t stores the energy. Anthelmintic drugs show paralytic action due to this they don’t have ability to uphold their position in gut6. One of the most common synthetic anthelmintic drug used is Albendazole. It acts by blocking the glucose uptake of larvae; thus, it decreases the level of glycogen in adult which leads to decrease in formation of ATP, due to this the worms get immobilized and leads to death7.

Need of Work

The synthetic antihelminth drugs are primarily used for treatment of helminthic infection caused by the various
species of helminthes. Ideally the anthelmintic agent should have large scale of action and should have effective curing ability with single therapeutic dose, without causing any harm to host8. The requirement for new and effective anthelmintic drug is massive, as the synthetic drugs used in the control of helminth is costly and the majority of them lose their efficiency in 15-20 years due to the problem of resistance5. Various synthetic medicine have been used for the treatment of helminthic infection like Albendazole, Benimidazole, Thiabendazole, Levamisole, Butamisole, Pyrantel, Morantel, Oxantel, Bephenium and Thenium. Anthelmintic drugs have various adverse drug reactions like toxocariasis, severe renal failure, sickness, vomiting, abdominal pain10-13. These side effects caused by synthetic anthelmintic drugs can be overcome by using herbal formulation, herbal formulations are safe to use and cost effective. In ancient times near to 3000 years ago in India when there are no synthetic medicines was developed then people used Ayurvedic plants14-16. The herbal drugs contain chemicals as alkaloids, glycoside, flavonoid, glycoside, carbohydrate and protein17. In phytochemical screening, Pisum sativum extract indicated the presence of therapeutically active chemical constituent’s flavonoids polyphenols alkaloids terpenoids and absence of saponin18. Pisum sativum contains various chemical constituents such as flavonoids, iso-flavonoids, anthocyanin and phenolic acids19-21. It also contains various nutrients like Protein, carbohydrate, starch, folate, vitamins as well as minerals20,21. Pisum sativum shows various pharmacological activities like Anticancer activity22,23 Anti-hyperglycaemic and anti-diabetic activity, Antioxidant activity, ACE inhibitors activity24-29.

MATERIALS AND METHODS

Plant Material

The plant material was gathered from nearby region of Pune, and was confirmed from branch of pharmacology AISSMS College of pharmacy, Pune. The pods of Pisum sativum were gathered in month of April. Collected plant pods were washed with water to eliminate soil and different contaminants. The pods were dried before preparation of extract.

Drugs and Chemicals

Table 1: Drugs and chemicals

| Sr.no | Reagent       |
|-------|---------------|
| 1     | Deionized water |
| 2     | Albendazole tablet |

METHODS

Preparation of aqueous extract of Pisum sativum30

Green peas (Pisum sativum L) were bought from a nearby shop in Pune, and straight away taken to the laboratory for in addition processing. The peas were rinsed very well with faucet water, dried with tissue paper and the seeds were isolate from the seed coat. The seed coats (the outer peel), which include nonedible component, were then cut into smaller portions and seed were triturated with mortar and pestle. Approximately 25 g of the outer peels was dipped in a 100 ml of deionized water in a 250 ml conical flask and heated for 10-15 minutes with non-stop stirring. This was then allowed to chill to room temperature and filtered via Whatman No.1 filter paper. The filtrate of Pisum sativum extract transferred in a neat and clear bottle and saved at 4°C till addition use.

Selection of the Experimental Model

Indian adult earthworms obtained from a neighborhood vendor were washed with normal saline to remove fecal matter. As per the experiment protocol earthworms of 8-10 cm length and 0.3-0.4 cm in width were used. Easy availability, anatomical and physiological similarities of earthworm with human intestinal round worm parasite was helpful to be used initially for in vitro evaluation (assay) of anthelmintic activity.

Experimental Design31

Test extract of pisum sativum was examined for anthelmintic activity using earthworms. Numerous concentrations of extract were tested using bioassay; determinations for time of paralysis and time of death were included in testing. The standard reference drug was Albendazole while saline water was taken as control. Prepared extracts and standard drug solution was placed in different petri plates in which later the earthworms were released as per group and different concentrations. Earthworms were grouped into five and all the solutions were freshly prepared in normal saline before commencement of experiment. Time taken for paralysis and death of worms were the two notable observations which time of paralysis was interpreted when no motility was seen except when the worms were vigorously disturbed. Interpretation of death was done when earthworms lost their mobility followed by fading away of their body colour.

Preliminary Phytochemical Analysis:32

The aqueous extract of Pisum sativum was obtained from the above procedure and then subjected to qualitative tests for the identification of various plant constituents like alkaloids, flavonoids, carbohydrates, glycosides, saponin, proteins, and steroids.

1. Detection of alkaloids

a) Mayer’s Test: 1-2 drops of Mayer’s reagent (Potassium mercuric iodide) treated with few ml filtrates. A creamy white/yellow precipitate formation indicates the presence of alkaloids31-33.

b) Wagner’s Test: 1-2 drops of Wagner’s reagent (Iodine in potassium iodide) treated with few ml filtrate. A brown/reddish precipitate formation indicates the presence of alkaloids.
To 2ml of extract add few drops of 0.5ml filtrate treated with 0.5ml solution. A creamy white precipitate formation indicates the presence of alkaloids.

d) Hager’s Test: 1-2 ml of Hager’s reagent (Saturated picric acid solution) treated with few ml filtrate. A reddish-brown precipitate formation indicates the presence of alkaloids.

2. Detection of flavonoids

a) Alkaline Reagent Test: 1 ml extract treated with 2 ml of 2% NaOH solution. An intense yellow color formation indicates the presence of flavonoids

b) Lead Acetate Test: 1ml plant extract treated with few drops of 10% lead acetate solution. A yellow precipitate formation indicates the presence of flavonoids

3. Detection of carbohydrates

In 5ml distilled water the extract was dissolved individually and filtrates. Filtrate was used to test for the presence of carbohydrate.

a) Molisch’s Test: 2ml filtrate treated with 2 drops of alcoholic alpha naphthol and 1ml conc. H2SO4 along the sides of the test tube. A violet ring formation at the junction indicates the presence of carbohydrates.

b) Benedict’s Test: 0.5ml filtrate treated with 0.5ml Benedict’s reagent and then it boiled for 2 minutes. A green/yellow/red color formation indicates the presence of reducing sugar.

4. Detection of glycosides

Dilute HCl used for hydrolysis of extract and then subjected to check for glycosides.

a) Modified Borntrager’s Test: Few ml of extract reacted with ferric chloride solution and immersed in boiling water for approximately 5 minutes. Cooled the mixture and extract with identical volumes of benzene. Separate the benzene layer and reacted with ammonia solution. Rose pink color produce in the ammonical layer shows the presence of anthranilic glycosides.

5. Detection of saponins

a) Hemolytic Test: Place one drop of blood on glass slide to which add test extract of Pisum sativum, formation of hemolytic zone takes place

b) Foam Test: 0.5 gm of extract was shaken with 2 ml of distilled water for 15 min. If foam produced is stable it indicates the presence of saponins.

6. Detection of proteins

a) Biuret’s Test: To 2ml of filtrate add 1 drop of copper sulphate, 1ml of 95% ethanol and KOH pellets pink to violet color formation in ethanolic layer indicates presence of proteins.

b) Millon’s Test: To 2ml of extract add few drops of millions reagent and heat, formation of white precipitate is observed indicating presence of proteins.

c) Xanthoprotein Test: To add few drops of con. Nitric acid, yellow coloration is seen indicating presence of proteins.

d) Ninhydrin Test: To 2ml of extract adds 2 drops of Ninhydrin solution, purple coloration is seen indicating presence of amino acids.

7. Detection of steroids

a) Salkowski reaction: Extract was shaken with chloroform and then 2ml con H2SO4 was added along sides of test tube, reddish-brown color formation indicated presence of terpenoids

b) Liebermann-Burchard: Extract of Pisum sativum was shaken with chloroform and then few drops of acetic anhydride were to the test tube followed by boiling in water bath and rapid cooling then add con H2SO4 along test tube, brown ring formation at junction of layers the upper later showing green coloration shows presence of steroids

8. Detection of phenols

Ferric Chloride Test: To extract of pism sativum add few drops of 5%ferric chloride solution, dark green to bluish black coloration seen indicating presence of phenols.

RESULTS AND DISCUSSION

The observations of preliminary phytochemical screening of aqueous extract of Pisum sativum are showed below. Pisum sativum extract gives positive results for presence of alkaloid, flavonoids, carbohydrate, saponins, steroids and protein.

Table 2: Preliminary phytochemical screening

| Sr no. | Phytochemical test | Result |
|--------|--------------------|--------|
| 1      | Test for alkaloids | Positive |
| 2      | Test for flavonoid | Positive |
| 3      | Test for carbohydrates | Positive |
| 4      | Test for glycoside | Negative |
| 5      | Test for saponin  | Positive |
| 6      | Test for protein  | Positive |
| 7      | Test for steroids | Positive |
| 8      | Test for phenols  | Negative |

Anthelmintic activity of aqueous extract of Pisum sativum analysis

Aqueous extract of Pisum sativum was given, which shows significant activity on earthworm. It was seen that When Control Group was compared with Positive Group and Test Group- I, II, III it showed significant (p<0.001) in paralytic condition and death. When Positive Group was compared with Test Group-III and Group-III it showed significant (p<0.001) in paralytic condition and death.
Table 3: Anthelmintic activity of aqueous extract of *Pisum sativum* analysis

| Treatment   | Concentration for (mg/ml) | Paralysis time min | Death time min |
|-------------|---------------------------|--------------------|----------------|
| Group I - Control | -                         | No paralysis       | No paralysis   |
| Group II - Albendazole (50mg/ml-Albendazole) | 40.17±2.30             | 48.50 ± 1.60       |
| Group III - Test I (5ml extract + 4ml Albendazole-50mg/ml) | 35.00±1.65             | 38.00 ± 1.71       |
| Group IV - Test 2 (10ml extract + 4ml Albendazole-200mg) | 21.83 ± 1.13            | 28.00 ± 2.63       |
| Group V - Test 3 (15ml extract + 4ml Albendazole-200mg) | 14.33 ± 1.30            | 19.00 ± 1.48       |

Values represent mean ± SEM; n=6; Analysis was performed using one way ANOVA Followed by Tukey’s multiple comparison tests. A p value less than 0.05 was considering as statistically significant. p value: a<0.05, b< 0.01, c<0.001 when compared with control. p< 0.05, q<0.01, r<0.001 when compared with positive control. x< 0.05, y< 0.01, z< 0.001 when compared with standard group.

**CONCLUSION**

Phytochemical analysis of the extracted revealed presence of phytoconstituents such as alkaloid, flavonoids, carbohydrates, glycoside, and protein. The present data indicate that aqueous extract of *Pisum sativum* is to be a safe anthelmintic effect and could be used as a part of therapy to treat parasitic infections of humans. Based on the findings of the present study it is concluded that, the aqueous extract of *Pisum sativum* found to have confirm their anthelmintic activity. We can conclude that aqueous extract of *Pisum sativum* exhibited most significant anthelmintic activity among the other Group. During study this plant showed very significant anthelmintic activity at Group- I, II, III measured by time taken for paralyse / death of the earth worms. Therefore, further study must be carried out so that the general people can get actual benefit from this important medicinal plant.

**Acknowledgements:** The authors would like to acknowledge Dr. Ashwini Madgulkar, Principal, AISSMS College of Pharmacy, Pune, for her encouragement and guidance.

**REFERENCES**

1. Devi K, Indumathy S, Rathinambal V, Uma S. Anthelmintic activity of Asta Churna. International Journal of Health Research. 2010; 2(1):101-104.10.4314/ijhr.v2i1.55399
2. Hotez P, Molyneux D, Fenwick A, Kumaressan J. Control of Neglected Tropical Diseases. New England Journal of Medicine. 2007;357(10):1018-1027.10.1056/nejmra064142
3. Kokate CK. Practical Pharmacognosy.4th ed. Delhi: Vallabh Prakashan; 1994.
4. Agharkar SP. Medicinal plants of Bombay presidency, PBI, Scientific Publishers, Jodhpur (India), 1991: 230.
5. Waller P, Thamsborg S. Nematode control in ‘green’ ruminant production systems. Trends in Parasitology. 2004;20(10):493-497.10.1016/j.pt.2004.07.012
6. Mutiarawati D. In Vitro Anthelmintic Activity of Acalypha Indica Leaves Extracts. Health Notions. 2020;4(3):94-99.10.33846/ijn40305
7. Nimila.IC, kumar.BA.In-Vitro Anthelmintic Activity of Mollugo Nudicaulis Lam, *Syzzygium cumini* Linn and *Hibiscus vitifolius* Linn on *Pheretima posthuma*. American Journal of PharmTech Research. 2020;10(6):1-10.10.46624/ajptr.2020.v10.i6.001
8. Patel J, Kumar G, Qureshi M, Jena P. Anthelmintic activity of Ethanolic extract of whole plant of *Eupatorium odoratum*. L. International journal of phytomedicine. 2010;2(2):127-132.10.5138/ijpm.2010.0975.0185.02020
9. Hammond JA, Fielding D, Bishop SC. Prospects for plant anthelmintics in tropical veterinary medicine. Vet Res Commun 1997;21:213-28.

10. Standen OD. Chemotherapy of Helminthic Infections. In: Experimental Chemotherapy, 1963; Vol. I: 701-892 [Edited by Schnitzer, R.J. & Hawking F.] Academic Press, New York and London

11. Edo-Taiwo O, Onyebuolise U. Parasitic helminth infection in anurans from Ozomo Wetland, Edo State, Nigeria. Tropical Freshwater Biology. 2020;29(1):99-110.10.4314/tfb.v29i1.7

12. Gathuma J, Mbaria J, Wanyama J, Kabubia H. Efficacy of Myrsine africana, Albizia anthelmintica and Hilderbrandtia sepalsosa herbal remedies against mixed natural sheep helminthiasis in Samburu district, Kenya. Journal of Ethnopharmacology. 2004;91(1):7-12. DOI 10.1016/j.ejep.2003.11.007

13. Ekeanyanwu R. C. In vitro anthelmintic potentials of Xylopia aethiopica and Monodora myristica from Nigeria. African Journal of Biochemistry Research. 2012; 6(9):1-10. 10.5897/abjr11.083

14. Devade OA, Londhe RD. Medicinal plants with Memory enhancing activity: review. J Pharm Adv Res, 2022; 5(2): 1452-1459

15. Devade O, Londhe R, Rathod N, Kupate J, Meshram N. Ayurvedic Remedies of Covid-19. International Journal of Pharmaceutical Sciences Research and Review. 2021; 70(2):58-64. 10.47583/iipssr.2021.v7i0i2.009

16. Mali KK, Sutar GV, Dias RJ, Devade OA. Evaluation of nootropic activity of Limonia acidissima against scopolamine-induced amnesic rats. turk j pharm sci 2021;18(1):3-9.10.4274/tjps.galenos.2019.30316

17. Devade O, Londhe R, Sokate N. A Review on: Polycystic Ovarian Disorder. Asian Journal of Research in Pharmaceutical Sciences. 2022;12(3):1-10.

18. Manke M, Dhawale S, Jankhande P. Helminthiaisis and medicinal plants: a review. Asian Pacific Journal of Tropical Disease. 2015;5(3):175-180.10.1016/s2222-1808(14)60648-4

19. Lustigman S, Prichard R, Gazzinelli A, Grant W. A Research Agenda for Helminthiasis of Humans: The Problem of Helminthiases. PLoS Neglected Tropical Diseases. 2012;6(4):e1582. 10.1371/journal.pntd.0001582

20. Githiori J, Höglund J, Waller P, Leyden Baker R. Evaluation of anthelmintic properties of extracts from some plants used as livestock dewormers by pastoralist and smallholder farmers in Kenya against Heligmosomoides polygyrus infections in mice. Veterinary Parasitology. 2003;118(3-4):215-226.10.1016/j.vetpar.2003.10.006

21. Iqbal Z, Lateef M, Ashraf M, Jabbar A. Anthelmintic activity of Artemisia brevifolia in sheep. Journal of Ethnopharmacology. 2004;93(23):265-268. 10.1016/j.jep.2004.03.046

22. Iqbal Z, Lateef M, Jabbar A, Muhammad G, Khan M. Anthelmintic activity of Calotropis procera (Ait.) Ait. F. flowers in sheep. Journal of Ethnopharmacology. 2005;102(2):256-261.10.1016/j.jep.2005.06.022

23. NWUDE N, IBRAHIM M. Plants used in traditional veterinary medical practice in Nigeria. Journal of Veterinary Pharmacology and Therapeutics. 1980;3(4):261-273.10.1111/j.1365-2885.1980.tb00491.x

24. Bell E. Phytochemical Dictionary of the Leguminosae, Volume 3, plants and their constituents 1051 pp.; Volume 2, chemical constituents 573 pp.: Compiled by I. W. Southon. Phytochemistry. 1995;38(4):1063. DOI 10.1016/0031-9422(95)0189-2

25. Waller PJ, Thamsborg SM. Nematode control in green ruminant Production systems. Trends Parasitol 2004;20:493-7

26. Ambriz D, Leyva N, Gutierrez E, Heredia J. Phenolic compounds: Natural alternative in inflammation treatment. A Review. Cogent Food &amp; Agriculture. 2016;2(1).10.1080/23311932.2015.1131412

27. Martens LG, Nilsen MM, Provan F. Pea hull fibre: novel and sustainable fibre with important health and functional properties. EC Nutrition 2017;10:139–148

28. Singh B, Singh J, Kaur A, Singh N. Phenolic composition and antioxidant potential of grain legume seeds: A review. Food Research International. 2017;101:1-16.1016/j.foodres.2017.09.026

29. Dahl W, Foster L, Tyler R. Review of the health benefits of peas (Pisum sativum L.). British Journal of Nutrition. 2012;108(5):S3-S10.10.1038/s0007114512000852

30. Stanisavljević N, Ilić M, Matić I, Jovanović Ž. Identification of Phenolic Compounds from Seed Coats of Differently Colored European Varieties of Pea (Pisum sativum L.) and Characterization of Their Antioxidant and In Vitro Anticancer Activities. Nutrition and Cancer. 2016;68(6):988-1000. 10.1080/01635581.2016.1190019

31. Mulla WA, Thorat VS, Patil RV, Burade KB. 2010, Anthelmintic activity of leaves of Alocasia indica Linn. International Journal of PharmTech Research; 2(1): 26-30

32. Kokate CK. Practical Pharmacognosy.4th ed. Delhi: VallabhPrakashan; 1994

33. Barbana C, Boye J. Angiotensin I-converting enzyme inhibitory activity of chickpea and peaprotein hydrolysates. Food Research International. 2010;43(6):1642-1649.10.1016/j.foodres.2010.05.003

34. Segura-Campos M, Chel-Guerrero L, Betancur-Ancona D. Purification of angiotensin I-converting enzyme inhibitory peptides from a cowpea (Vigna unguiculata) enzymatic hydrolysate. Process Biochemistry. 2011;46(4):864-872.10.1016/j.procbio.2010.12.008

35. Gibbs B, Zougman A, Masse R, Mulligan C. Production and characterization of bioactive peptides from soy hydrolysate and soy-fermented food. Food Research International. 2004;37(2):123-131.10.1016/j.foodres.2003.09.010

36. Manke M, Dhawale S, Jankhande P. Anthelmintic potential of Helicteres isora bark extract against Pheretima posthum. Asian Pacific Journal of Tropical Disease. 2015;5(4):313-315.10.1016/s2222-1808(14)60789-1

37. Bagheri H, Simiand E, Montastruc J, Magnaval J. Adverse Drug Reactions to Anthelmintics. Drug Reactions to Anthelmintics. 2012; Vol. I: 701 pp.; Volume 2, chemical constituents 573 pp.: Compiled by I. W. Southon. Phytochemistry. 1995;38(4):1063. DOI 10.1016/0031-9422(95)0189-2

38. Hadrich F, Arbi M, Boukhris M. Valorization of the Peel of Pea: Pisum sativum by Evaluation of Its Antioxidant and...
Antimicrobial Activities. Journal of Oleo Science. 2014;63(11):1177-1183.10.5650/jos.ess14107

39. Taubeneck U. J. D. Bu’lock, L. J. Nisbet and D. J. Winstanley (Editors), Bioactive Microbial Products: Search and Discovery. VII+ 148 S., 35 Abb., 39 Tab. London-New York-Paris-San Diego-San Francisco-Sao Paulo-Sydney-Tokyo-Toronto 1982. Academic Press. £ 20.50. Zeitschrift für allgemeine Mikrobiologie. 1983;23(2):143-143.10.1002/jobm.19830230208

40. Singh B, Singh J, Kaur A, Singh N. Phenolic composition, antioxidant potential and health benefits of citrus peel. Food Research International. 2020;132:10 9114.10.1016/j.foodres.2020.109114

41. Davis D, Arnold C, McCallum I. Nutritional value of feed peas (Pisum sativum) in practical diet formulations for Litopenaeus vannamei. Aquaculture Nutrition. 2002;8(2):87-94. 10.1046/j.1365-2095.2002.00194.x

42. Stanisavljevic N, Ilic M, Jovanovic Z, Cupic T. Identification of seed coat phenolic compounds from differently colored pea varieties and characterization of their antioxidant activity. Archives of Biological Sciences. 2015;67(3):829-840.10.2298/abs141204042s

43. Poralijan V, Shokuh Rad A. Extraction of Eugenol from Carnation: A Quantitative and Qualitative Analysis by Aqueous and Ethanolic Solvents. Journal of Essential Oil Bearing Plants. 2016;19(6):1495-1502.10.1080/0972060x.2016.1211962

44. Raaman N. Phytochemical Techniques. New India Publishing Agency, New Delhi, 2006, 19-24

45. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A Review. International Pharmaceutica Scientia. 2011;1(1):98-106.

46. Joshi A, Bhobe M, Saatarkar A. Phytochemical investigation of the roots of Grewia microcos Linn. J. Chem. Pharm. 2013;5:80-87.