Preliminary Phytochemical Screening and Standardization of *Pisum sativum*

Dr. R. Manohar[1], T.Ananda Lahari[2], Y.Upendra[3], Bandi Mrudula[4]

1. Professor, P. Rami Reddy Memorial College of Pharmacy, Kadapa - 516 003, Andhra Pradesh, India. reddy.manohar1981@gmail.com
2. P. Rami Reddy Memorial College of Pharmacy, Kadapa - 516 003, Andhra Pradesh, India.tanandalahari2@gmail.com
3. P. Rami Reddy Memorial College of Pharmacy, Kadapa - 516 003, Andhra Pradesh, India.yupendra953@gmail.com
4. P. Rami Reddy Memorial College of Pharmacy, Kadapa - 516 003, Andhra Pradesh, India. bandimrudulareddy2@gmail.com

Corresponding author:
Dr. R.Manohar
Professor & HOD
Department of Pharmacology
P.Rami Reddy Memorial College of Pharmacy, Kadapa.
Andhra Pradesh (State), India.
Email: reddy.manohar1981@gmail
Contact: +91 9963085878

Abstract:

Traditional medicines are widely perceived as natural and safe, that is, not toxic. This is not necessarily true, especially when herbs are taken with prescription drugs, over-the-counter medications, or other herbs, as is very common. World Health Organization determined explicit rules for the assessment of the wellbeing, adequacy and quality of herbal drugs. The current examination was attempted to the standardization of seeds of Pisum sativumand to assess the fundamental phytochemical investigation. In this investigation the dried seeds ofPisum sativumwere assessed for organoleptic characters, physicochemical boundaries of pH, loss on drying, ash values and fundamental phytochemical examination for distinguishing of chemical constituents. The information acquired by the current examination shows all the qualities within the determination of WHO and all these may accommodate in distinguishing of this therapeutic plant and may likewise supportive in forestalling its debasement.

Keywords: *Pisum sativum, Preliminary phytochemical screening, Standardization.*

Introduction

The emphasis on the use of medicinal plants had been placed on the treatment rather than prevention of diseases. However, there exists in the literature considerable report in recent times on research work on the use of medicinal plants and their constituents in disease prevention. A World Health Organization (WHO) Expert Group defined Traditional Medicine as the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental, or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing [1].

Furthermore, traditional medicines are widely perceived as natural and safe, that is, not toxic. This is not necessarily true, especially when herbs are taken with prescription drugs, over-the-counter medications, or other herbs, as is very common [2].

Legume or pulse is one of the traditional medicines used globally because it has the amount of nutritional substances and has the efficiency of therapeutic treatments [3]. Pea is one of the major food legumes that can grow in different regions, and it ranks the fourth in world food legume productions next to soybean, peanut, and dry bean [4].Seed and sprout of pea have become increasingly consumed because people concern about their health problem by changing dietary habits [5].The present review explores scientific evidences to provide updated information about the properties of green pea or garden pea (*Pisum sativum*) [6].

Materials and methods

Collection and authentication of seeds

The fresh seeds of *Pisum sativum* (Fabaceae) were pull together from Kadapa district and authentified by Dr. K. Madava Chetty, Professor, Department of Botany, Sri Venkateswara University, Tirupathi. Andhra Pradesh, India. Voucher specimens (No:PS - 1520) for this plant has been kept in the P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India.

Plant description

Pisum sativum is an annual herbaceous legume in the family Fabaceae grown for its edible seeds and seedpods. The pea plant can be bushy or climbing, with slender stems which attach to a substrate using tendrils. Each leaf has 1–3 pairs of oval leaflets and can reach 1–6 cm in length. The plant produces white, red or purple flowers and swollen or compressed green seedpods which can be straight or curved. The pods can range in size from 4 to 15 cm long and 1.5–2.5 cm wide. Each pod contains between 2 and 10 seeds, or peas. The pea plant is an annual plant, surviving only one growing season and can reach 30–150 cm in height. Pea may also be referred to as garden pea, English pea or green pea and likely originates from Southwest Asia.


Fig. 1: Seeds of *Pisum sativum*

Propagation

Basic Requirements

Peas are cool-season crops and should be grown in early Spring or late summer to avoid high summer temperatures. Peas grow best at temperatures between 15 and 24°C (60–75°F) and are tolerant of frost down to -6.5°C (20°F) although they are generally less sensitive to Spring frost. Pea plants will grow best in soils rich in organic matter with a pH between 5.5 and 7. They should be planted in a well-draining soil or raised bed as they do not tolerate too much moisture. Peas perform best in full sunlight although they are also tolerant of partial shade. Peas should be direct seeded in the garden in Spring as soon as soil is workable and its temperature is above 4.4°C (40°F), or in late summer 8–10 weeks before the first frost date. The optimum soil temperature for germination is 10–25°C (50–77°F), lower temperatures will slow germination. Seeds should be planted 2.5–5.0 cm (1–2 in) with shallow planting beneficial in soils which are cool and wet. Seeds should be sown 2.5–10.0 cm (1–4 in) apart allowing 45 cm (~18 in) between rows. Seedling should emerge in 9–13 days at a soil temperature of 15.5°C (60°F) and do not require thinning. General care and maintainance Tall, vining pea varieties require a trellis to support their growth. Trellises can be made easily out of chicken wire or lengths of string and the plants will begin to grow around them, using tendrils to grip around the support. If growing a variety that requires trellising then the row spacing should be adjusted accordingly. Cool season crops are often attacked by powdery mildew and where the disease is known to be problematic, resistant varieties should be planted [7].

Taxonomical Classification [8]

Kingdom	Plantae
Phylum	Spermatophyta
Super Division	Embryophyta
Division	Tracheophyta
Sub division	spermatophyta
Class	*Magnoliopsida*
Super order	Rosanae

Order	Fabales
Family	Fabaceae
Genus	Pisum
Species	Sativum

Table 1: Taxonomical classification of *Pisum sativum*

Phytochemical Substances

The active phytochemical substances of *Pisum sativum* are as follows:Asparaginase; flavonoids including apigenin, daidzein, genistein, and kaempferol; lectin; phenolic compounds including caffeic, catechin, coumaric acids, gentisic acids, ferulic, protocatechuic, and vanillic acids; pisatin and an allelopathic active substances; proanthocyanidin; saponins; steroid phytohormone including brassinosteroid; and tannins[9].

Preparation of powdered seed material

The gathered seeds of *Pisum sativum* were separately washed with running water, cut into little pieces and shade dried at room temperature to maintain a strategic distance from loss of phytoconstituents of plant. The total shade dried materials pounded for powder and sieved up to 80 meshes. At that point it was homogenized to fine powder and put away in air-tight compartment for additional considers.

Organoleptic evaluation

The seeds powder of plant *Pisum sativum* were evaluated for its organoleptic properties such as appearance, color, taste, touch and texture [10].

Physicochemical investigations

To standardize the chose medicinal seeds of plants *Pisum sativum* were oppressed for assurance of physicochemical parameters, for example, pH value in 1% and 10% solution, loss on drying, ash values, aqueous, and alcoholic extractive qualities were done by the strategies suggested by the World Health Organization [11].

Determination of pH range

The pH of various details in 1% w/v (1g: 100ml) and 10% w/v (10g: 100ml) of water solvent parts of entire plants powder of plant *Pisum sativum* were resolved utilizing standard straight forward glass electrode pH meter [12].

Loss on drying / moisture content (Gravimetric determination)

Independently place about 1.0 g of seeds powder of the plant *Pisum sativum*, in a precisely weighed moisture disc. For estimation of loss on drying, it was dried at 105°C for 5 hours in oven preheated to as indicated temperature, cooled in a desiccators for 30 minutes, and weighed immediately. The loss of weight was determined as the substance of in mg per g of airdried material.

Determination of total ash

Two grams of seeds powder of *Pisum sativum* were independently positioned in an antecedently ignited (350°C for 60 minutes) and tarred crucible precisely gauged. Dried material was spread in an even layer in the crucible and the material ignited by continuously expanding the warmth to 550°C for 5 hours in a muffle furnace until it was white, contending the nonattendance of carbon. Cooled in a desiccator and gauged. Total ash content was determined in mg per g of air-dried material.

Determination of acid insoluble ash

Continue according to the means referenced in the methodology for determination of total ash estimation of crude drug seeds of *Pisum sativum* and further as follows. Twenty-five (25) ml of hydrochloric acid (~70g/l) test solution was added to the crucible containing the total ash, secured with a watch glass and boiled tenderly for 5 minutes. The watch glass was flushed with 5 ml of high temp water and this liquid added to the crucible. The insoluble matter was gathered on anash less filter paper (Whatmann - 41) and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble issue was moved to the original crucible, ignited by step by step expanding the heat to 550°C for 3 hours in a muffle furnace to steady weight. Permitted the residue to cool in an appropriate desiccator for 30 minutes, and afterward weighed immediately. Acid insoluble ash content was determined as mg per g of air-dried material.

Determination of water-soluble ash

Twenty five (25) ml of water was added to the crucible containing the total ash, secured with a watch glass and boiled delicately for 5 minutes. Insoluble matter was gathered on a debris less filter paper. Washed with boiling water and ignited in a crucible for 15 minutes at a temperature not surpassing 450°C in a muffle furnace. Permitted the residue to cool in appropriate desiccators for 30 minutes, and afterward weighed immediately. The weight of the residue was deducted in mg from the weight of total ash. Water soluble ash content was determined as mg per g of air dried material.

Determination of sulfated ash

Ignited an appropriate crucible (silica) at 550°C to 650°C for 30 minutes, cooled the crucible in desiccators (silica gel) and weighed it precisely. One gram of seeds powder of the plant *Pisum sativum* were set independently in a formerly ignitedcrucible, lighted delicately from the start, until the substance was completely white. Cooled and moistened the sample with a limited quantity (normally 1 ml) of sulfuric acid (1760 g/l) test solution, heated delicately at a temperature as low as practicable until the sample is altogether charred. In the wake of cooling, moistened the residue with a modest quantity (normally 1 ml) of sulfuric acid (1760 g/l) test solution, warmed delicately until white fumes were didn’t advance anymore, and ignited at 800°C + 25°C until the residue was totally burned. Guarantee that flames were not delivered whenever during the system. Cooled the crucible in a desiccator (silica gel), weighed precisely. This was repeated until the sample arrives at a consistent weight and determined the percentage of residue [13].

Determination of hot water and ethanol extractable matter

Independently place about 4.0 g of *Pisum sativum* seeds powder in a precisely weighed, glass stoppered conical flask. For estimation of hot water extractable matter, 100 ml of distilled water was added to the flask and weighed to get the total weight including the flask. The substance was shaken well and permitted to represent 60 minutes. A reflux condenser was joined to the carafe and bubbled delicately for 60 minutes; cooled and weighed. The flask was reset to the original total weight with distilled water and it was shaken well and filtered rapidly through a dry filter. At that point, 25 ml of the filtrate was moved to a precisely weighed, tarred flat bottomed dish (Petri dish) and evaporated to dryness on a water bath. At long last, it was dried at 105°C for 6 hours in anoven, cooled in desiccators for 30 minutes and weighed immediately. Same method was followed utilizing ethanol rather than distilled water to decide extractable issue in ethanol. The extractable issue was determined as the substance of in mg per g of air dried material.

Preparation of extract

Pisum sativum seed powder were extracted with ethanol in a Soxhlet apparatus in batches of 500 gm each. The overabundance solvent was expelled from extract utilizing a rotary vacuum evaporator and later on concentrated on a water bath. The rate yield of the extract was determined. At last dried extract was put away in desiccators for preliminary phytochemical screening [14].

Preliminary phytochemical screening

Ethanolextract of *Pisum sativum* seeds were subject to qualitative chemical test so as to determine the presence or absence of phytochemical constituents such as alkaloids, glycosides, flavonoids, starches, saponins, tannins, terpinoids, steroids, reducing sugars, proteins, amino acids, fats and oils [15,16 &17].

Test for alkaloids

Mayer's test: One ml of each extract was acidified with 0.2 ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent was added. Arrangement of yellow or white coloured precipitate demonstrates the nearness of alkaloids.

Dragendroff's test: Extracts were added to 1 ml of dilute hydrochloric acid and 0.1 ml of Dragendroff's reagent (Solution of Potassium Bismuth Iodide). Formation of orange brown coloured precipitate demonstrates the presence of alkaloids.

Test for reducing sugars

Fehling's test: To 5 ml of extracts mixed with same quantity of Fehling's solution A & B were added and boiled on water batch for approximately 10 minutes. Formation of brick red coloured precipitate shows the positive for reducing sugars.

Benedict's test: To 5 ml of extracts mixed with Benedict's reagent in test tube and boiled for few minutes. Appearance of brick red precipitate conform the bearing of reducing sugars.

Test for steroids:

Libermann-Burchard test:10 mg of extract was dissolved in 1ml of chloroform ($CHCl_3$). To this 1 ml of acetic anhydride was added following the addition of 2ml of concentrated sulphuric acid, a reddish violet color developed, indicating the bearing of steroid [18].

Salkowski test:1 ml of concentrated sulphuric acid (Conc. H_2SO_4) was added to 10 mg of extract dissolved in 1 ml of chloroform. A reddish-blue color exhibited by chloroform layer and green fluorescence by the acid layer suggested the bearing of steroids.

Test for protein and amino acids

Biuret test: Few ml of the extract were mixed with 1 ml of 40% NaOH and two drops of 1% copper sulphate. Appearance of violet colour demonstrates the bearing of proteins.

Ninhydrin test: Few drops of freshly prepared 0.2% Ninhydrin reagent were added to extract and heated for few minutes. Development of blue color designates the bearing of proteins or peptides or amino acids.

Xanthoprotein test: White precipitate was formed on treating the extract with 1 ml concentrated nitric acid. Further, boiled and cooled and the ammonia was added. Appearing of orange color designates the bearing of aromatic amino acids.

Test for tannins

Small quantity of the extract was permit to react with 1 ml of 5% ferric chloride solution. Greenish black coloration designates the bearing of tannins.Few ml of the extract were treated with 1 ml of 10% lead acetate solution. Development of white color precipitate indicates the bearing of tannins.

Test for flavonoids

5 ml of the extracts were hydrolyzed with 10 % v/v sulphuric acid and cooled. At that point, it was extracted with diethyl ether and partitioned into three bits in three separate test tubes. 1 ml of diluted sodium carbonate, 1 ml of 0.1N sodium hydroxide, and 1 ml of strong ammonia solution were added to the main, second and third test tubes separately. In each test tube, advancement of yellow shading exhibited the nearness of flavonoids.

Shinoda's test

The extracts were broken up in 2 ml of alcohol, to which hardly any magnesium turnings were included trailed by concentrated hydrochloric acid drop wise and warmed. Appearance of magenta shading shows the nearness of flavonoids.

Test for gums and mucilage

Molisch's test: To 2 ml of the extract solution was added to 2 ml of concentrated sulphuric acid and then treated with molisch's reagent (15% ethanolic α-naphthol). Formation of reddish violet ring at the junction of two layers shows the bearing of gums and mucilage.

Test for glycosides

Legal test: Small amount of extracts were dissolved in pyridine and made alkaline by adding sodium nitropruside. Pink red color was produced.

Baljet test: By the addition of sodium picrate solution the extract yellow to orange color was produced.

Borntrager's test: Few ml of dilute sulphuric acid added to plant extracts. Boiled, filtered and extracted the filtrate with either or chloroform. Then organic layer was separated to which ammonia was added, pink red color was give rise in organic layer.

Test for saponins

Foam test: Around 1 ml of the extract was weakened to 20 ml with distilled water and shaken well in a graduated chamber for 15 minutes. The development of foam in the upper part of the test tube showed the nearness of saponins.

Test for triterpinoids

In the test tube the extract was added to 2 or 3 granules of tin and dissolved in 2 ml of thionyl chloride solution. Appearance of pink color shows the appearing of triterpinoids.

Test for fats and oils

Spot test: A drop of concentrated extract was pressed in between two filter papers and kept undisturbed. Oil stain on the paper demonstrates the bearing of oils and fats.

Results and discussion

Organoleptic evaluation

Organoleptic assessment is a qualitative strategy utilized dependent on the investigation of morphological and sensory profiles of entire medications[19]. As found in table 2, the seeds of *Pisum sativum* showed up as raw powder and the ethanol extract were in semisolid structure. The seed powder is course in nature and the extract were in smooth. Color of the powder is showed up as greenish colored and while ethanolextractwere showed up as green. Taste and the odour of powder and the extract show comparative which are bitter and characteristic individually.

Plant	Parameters	Raw	Ethanol extract
Pisum sativum	Appearance	Powder	Semisolid
	Touch	Coarse	Smooth
	Color	Greenish	Greenish
	Taste	Bitter	Bitter
	Odour	Characteristic	Characteristic

Table 2: Organoleptic characteristics of *Pisum sativum*

% of Yield

According to organized in table 3, the average yield (% w/w) of seeds of*Pisum sativum*was found as 2.8 during extraction with ethanol.

Plant	**Type of extract**	**Amount of extract (gm)**	**% Yield (w/w)**
Pisum sativum	Ethanol	15	2.8

Table 3: % Yield of seeds of *Pisum sativum*

Physicochemical investigation

Assessment of physicochemical characteristics of the seeds powder is a significant boundary in identifying the adulterations of drugs. Physicochemical parameters of seeds powder of *Pisum sativum* were anticipated dependent on the techniques suggested by World Health Organization (WHO) and the outcomes got are arranged in Table 4. The pH of 1% w/v and 10% w/v solutions were seen as (4.34± 0.05) and (4.65 ± 0.06) separately for plant *Pisum sativum*. These qualities were indicated very little contrast in the pH of water solvent bits of seeds powder.

Percentage weight reduction on drying or moisture content qualities were seen as (7.73 ± 0.31)seeds of *Pisum sativum*. This is particularly significant for materials that ingest moisture effectively or break down rapidly in nearness of water. The less estimation of moisture substance of medications could forestall content bacterial, parasitic or yeast development through storage [20 & 21].

The ash values were controlled by various techniques, which incorporate total ash, acid insoluble ash, water soluble ash and sulfated ash value. The complete ash estimations of total ash, acid insoluble ash, water soluble ash and sulfated ash values were seen as (12.38 ± 0.34), (2.85 ± 0.20), (2.21± 0.11) and (1.46± 0.04) for *Pisum sativum* seeds. The residue staying after burning of seed material is the ash substance or debris value, which just speaks to inorganic salts as contaminated by holding fast to it or normally happening in crudedrug or purposely added to it.

The solubility percentage of seeds ofplant *Pisum sativum*in aqueous hot extraction was higher (26.75 ± 1.25), when compared with ethanolic hot extraction (19.26 ± 1.26) of *Pisum sativum* [22].

Table 4: Physicochemical parameters of *Pisum sativum*

Parameters	Values of Physicochemical Parameters of *Pisum sativum*
pH of 1% w/v formulation solution [H^+]	4.34 ± 0.05
pH of 10% w/v formulation solution [H^+]	4.65 ± 0.06
Loss on drying (mg/g)	7.73 ± 0.31
Total ash value (mg/g)	12.38 ± 0.34
Acid insoluble ash (mg/g)	2.85 ± 0.20

Water soluble ash (mg/g)	2.21 ± 0.11
Sulfated ash value (% of residue)	1.46 ± 0.04
Water soluble (hot) extractive value(mg/g)	26.75 ± 1.25
Ethanol soluble (hot) extractive value(mg/g)	19.26 ± 1.26

Values are expressed as mean% ± SEM, except pH values are expressed as mean ± SEM.

Preliminary phytochemical screening

Ethanol extract of seeds powder of *Pisum sativum* were exposed to preliminary phytochemical investigation to test for appearance or nonappearance of different phytoconstituents and the outcomes acquired are outlined inTable 5. The seeds of the plant had a rich ownership for phytochemicals like alkaloids, reducing sugars, proteins and amino acids, flavonoids, glycosides, saponins and fats and oils in ethanol extract. Though steroids, tannins, gums and mucilage, triterpinoids were missing.

Table 5: Phytochemical constituents

Phytochemical constituent	Tests performed	Ethanol extract of*Pisum sativum* (seeds)
Test for alkaloids	Mayer's Test	+
	Dragendroff's Test	+
Test for reducing sugars	Fehling's Test	+
	Benedict's Test	+
Test for steroids	Libermann-Burchard Test	-
	Salkowski Test	-
Test for protein and amino acids	Biuret Test	+
	Ninhydrin Test	+
	Xanthoprotein Test	+
Test for tannins	Extract + Ferric chloride	-
	Extract + Lead acetate	-
Test for flavonoids	With NaOH	+
	Shinoda's test	+
Test for gums and mucilage	Molisch's Test	-
Test for glycosides	Legal test	+
	Baljet test	-
	Borntrager's test	+
Test for saponins	Foam test	+
Test for triterpinoids	Tin & thionylchloride test	-
Test for fats and oils	Spot test	+

(+) presence, (-) absence

Conclusion:

Medicinal plants represent a novel alternative and preferred treatment to handle ailments with no satisfactory remedy. For a long time, humans use herbal medicines to manage different diseases. Herbal drug development is possible only through the development of standardized herbal products with reference to their active phytoconstituents present for commercialization, correct identification and supply of raw materials and to avoid adulteration. The current examination was attempted totally to standardize the seeds of *Pisum sativum* by assessing its organoleptic, physicochemical and preliminary phytochemical boundaries according to world health organization (WHO). The data created by the current investigations will give information and which is helpful for distinguishing proof and also may helpful for forestalling its adulteration.

Acknowledgements

The authors are appreciative to the management ofP. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India, for giving vital offices to complete the research work.

Conflict of Interest

The authors declare that there was no conflict of interest in this research.

References

1. Abayomi Sofowora·EyitopeOgunbodede and Adedeji Onayade, "African journal of Traditional Complementary and Alternative Medicines"2013, 10(5),pp. 210–229.

2. *Canter and Ernst 2004,"African journal of Traditional Complementary and Alternative Medicines,2013, 10(5),pp. 210–229.*

3. Schneider AV,Br J Nutr," Overview of the market and consumption of pulses in Europe", 2002;88(Suppl 3):pp.243–50.

4. Vohra K, Dureja H, Garg V, "J Pharmacogn Nat Prod"An insight of pulses From food to cancer treatment, 2015,pp.1,108.

5. Vidal-Valverde C, Frias J, Hernandex A, Martin-Alvarez P, Sierra I, Rodriquez C, "JSci Food Agric"Assessment of nutritional compounds and antinutritional factors in pea (Pisum sativum) seeds,2003,pp.298–306.

6. Chon SU,"Curr Pharm Des",Total polyphenols and bioactivity of seeds and sprouts in several legume, 2013,pp.112–24.

7. *RunchanaRungruangmaitree and WanneeJiraungkoorskul ,"Pharmacogn Rev",2017 Jan-Jun, 11(21),pp.39–42[Accessed 05 March 15].*

8. Renu G, Sanjana JuliasThilakar, Narasimhan D, Centre for Floristic Research, Department of Botany, Madras Christian College, Tambaram,"Integrated Taxonomic Information System (ITIS)","Bougainvillea spectabilis Willd", Taxonomic Serial No:895413, Geological Survey, VA, USA 2016.

9.Chagas E, Sodek L,"Braz Arch Biol Technol" Purification and properties of asparaginase from the testa of immatureseeds of pea (Pisum sativum L.)2001,pp.239-45.

10.Siddiqui, Hakim M.A, Format for the pharmacopoeial analytical standards of compound formulation, workshop on standardization of Unani drugs, anuary, Central Council for Research in Unani Medicine (CCRUM), New Delhi, (appendix), 1995,pp.24-25.

11.World Health Organization, (WHO), Organization Mondiale De La Sante, Quality control methods for plant materials, 559, Rev. 1, Original English, 1998,pp.08-67.

12.Neeraj Choudhary, Bhupinder Singh S,"Journal of Pharmaceutical Education and Research", An overview of advances in the standardization of herbal drugs, 2011, 2(2),pp.55-70.

13.World Health Organization, (WHO),Final text for revision of The International Pharmacopoeia,Sulfated Ash,Document QAS/11.401 Final, 20s12,pp. 1-2.

14.Lachman and Lieberman, "The Theory and Practice of Industrial Pharmacy", 3rd Edn, CBS Publishers and Distributors,2007,pp.150-153.

15.Nidhi Saxena, Shrivastava PN, Saxena RC,"International Journal of Pharmaceutical Science and Research" Preliminary Physico-Phytochemical Study of stembark of Alstoniascholaris (L), 2012, 3(4),pp.1071-1075.

16.Kokate CK, Khandelwal KR, Pawer AP, Gokhale SB,NiraliPrakashanPune"Practical Pharmacognosy", 1995,3rd ed: pp.137.

17. Daphne Evans. Edinburgh London New York Philadelphia St Louis Sydney Toronto , "Trease and Evans" Phytochemistry, In Pharmacognosy, 2009, 16th ed: pp.133-168.

18.Mukherjee PK,"Quality Control of Herbal Drugs - An Approach to Evaluation of Botanicals", Business Horizons, New Delhi, India, 2002 pp.560–567.

19.Kokate CK, Purohit AP, Gokhale SB,NiraliPrakashan, "Pharmacognosy", 2006,34th ed: pp.145-151.

20.Manoj Kumar P, Singh GN, Rajeev Kr Sharma, Sneh Lata,"International Journal of Pharmaceutical Science and Research",Standardization of YakritPlihantakChurna, An Ayurvedic Poly herbal formulation, 2012, 3(1),pp.171-176.

21.Bhattacharya S, Kamaruz Zaman. Md,"International Journal of PharmTechResearch"Pharmacognostical Evaluation of Zanthoxylum nitidum Bark, 2009, 1(2),pp.292-298.

22.Shwetajain, Chanderachud Sharma, Pankaj Khatri, Atul Jain, Ankur Vaidya,"International Journal of Pharmacy and Pharmaceutical Sciences",Pharmacognostic and Phyto chemical Investigations of the Leaves of Zizyphusxylopyrus (Retz) Willd, 2011, Vol. 3, Issue 2,pp.122-125.