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PHYTOCHEMICAL ANALYSIS OF LABLAB PURPUREUS L. SEED EXTRACTS AND LEAVES

[1]Usha Vishwakarma, [2]Kumar Surayanshu, [3]Harsh Badode, [4]Gulshan Dhakad, [5]Hariom Parihar, [6]Jayash kumar underiya, [7]Dr. Jagdish Rathi

[1,2,3,4,5,6,7]NRI INSTITUTE OF PHARMACEUTICAL SCIENCES, BHOPAL, M.P.

Correpondence Author

[1]Usha vishwakarma

ABSTRACT: The aim of present study is phytochemical analysis of Lablab Purpureus L. Seed Extracts And Leaves. Firstly extraction done for different ingredients by solvent action. After that phytochemical analysis done for presence of various active constituents present in extracts. Lablab purpureus L. Belongs to the family fabaceae, commonly called as hyacinth bean, Dolichos bean, Seim bean, Egyptian bean, Kidney bean and Indian bean. Hyacinthis atropical perennial twinning plant.Flowers appear in clusters with purple or white colour,which develop into flat, thick, slightly curved green pods with pointing ends. In each pod generally six seeds are present, which are mottled, mostly oval in shape. In 100 grams of Dolichos bean contain proteins, carbohydrates, dietary fibres, vitamins A, B complex vitamins and minerals like Ca, Fe, Cu, Se, Mg, K and Zn along with bioactive Phytochemical compounds.

KEYWORDS: Extract, Drug, Phytochemical, Dosage form, Evaluation

INTRODUCTION

Lablab purpureus L. Belongs to the family fabaceae, commonly called as hyacinth bean, Dolichos bean, Seim bean, Egyptian bean, Kidney bean and Indian bean. Hyacinthisatropicalperennialtwinningplant.Flowersappearinclusterswithpurple or white colour, which develop into flat, thick, slightly curved green pods with pointing ends. In each pod generally six seeds are present, which are mottled, mostly oval in shape. In 100 grams of Dolichos bean contain proteins, carbohydrates, dietary fibres, vitamins A, B complex vitamins and minerals like Ca, Fe,Cu,Se,Mg, Kand Zn along with bioactive Phytochemical compounds. The present study deals with the phytochemical analysis of Dolichos bean !1)

The plant can show the pharmacological effects to the diseases of anti- microbial, antioxidant, anticancer, hypo-lipidemic, central nervous system, cardiovascular, respiratory, immunological, anti-inflammatory, analgesic, anti-pyretic, cytotoxic, insecticidal, anti-diabetic, anti-lithiatic, anemic diseases. The present study which deals with the phytochemical analysis of Lablab purpureus L seed extracts.

Sample preparation: Field seed samples collected from the Karimnagar District, Telangana, India. Seeds were washed many times and shadow dried. Seeds were subjected to pulverization to get coarse powder. It was stored in airtight container for further use. Solvents like Methanol and Water were subjected to soxhlet extraction. For every solvent 250 mg of seed powder was used against 150 ml of solvent. Fractions were collected after some rounds of successful extraction in the soxhlet apparatus (2).

Lablab purpureus (lablabbeans) is an under-utilized legumethathas potential ashuman food (Bawaet al., 2003). It is alegume that thrives well during the dry season between November and February in Northern Nigeria. It is drought resistant and is usually sown after the normal cropping season, thereby acting as a buffer crop for ruminant feeding during the dry season (Adu et al., 1992). L. purpureus is reported to have certain medicinal properties (Handa et al.,1989; Adeleke et al., 2012). Phytochemicals include secondary metabolites of plants, and other antinutritional factors,which are reported to be pharmacologically active (Zank, 1991). Several reports have provided evidence for the pharmacological effects of plant phytochemicals. Tannins are reported to have anthelmintic effects (Molanetal.,2000a, b) and useful in the treatment of inflamed or ulcerated tissues and they also have remarkable activities in the cancer prevention and anticancer activity (Akinpelu et al., 2009). Flavonoids, phenols and saponins. have been reported to exhibit their actions through effects on membrane permeability, antioxidative action and anti-inflammatory effects (Olayinka and Okoh, 2010).Many triterpene saponins and their aglycones have varied uses including anti-inflammatory, antipyretic, fibrinolytic, analgesic, anti- ulcerogenic, anti-oedema and antimicrobial agents (Hostettmann and Martson, 1995; Soetanetal.,2006; Ndukwe et al., 2007). Alkaloids are haemolytically active, toxicto micro-organisms and are widely used as therapeutic agents in the management of cancer (Viji and Parvatham, 2011). Glycosides are reported to inhibit tumour growth and to also protect against gastrointestinal infections (Adeshina et al., 2010). Terpenoids have been reported to be active against bacteria, fungi, protozoa and virus (Maiyo et al.,2010). El-Mahmood et al. (2008) linked antimicrobial properties of plants to bioactive secondary metabolites (saponins, tannins, alkaloids, flavonoids, phenols,

glycosides and diterpenes).

Due to the discovery of the beneficial effects of phytochemicals in food plants, this study was designed to evaluate the concentration of various phytochemicals in L. purpureus, which could be a guide to ascertain their pharmacological potentials. The phytochemicals to be evaluated are trypsin inhibitors, haemagglutinins, cyanogenic glycosides, oxalates, phytates, tannins, saponins and alkaloids.

Materials and Methods: All the extracts were used for phytochemical analysis. According to the test of content,test sample varies by following standard protocols and the results were listed in the tabular form.

Test for Reducing sugars: The extracts were treated with 5.0 ml of Fehling'ssolution and kept in boiling water bath. The formation of yellow or red colour precipitate indicates the presence of reducing sugars. (3).

Test for phenols: 2% FeCl3 was added to the 1ml of sample formed blue black colouration. This showed positive result for phenols. (4)

Test for steroids: 1 ml of the extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube.The upper layer turned red and the sulphuric acid layer formed fluorescent green with yellow. This indicated the presence of steroids. (5).

Test for Alkaloids: For the detection of presence of Alkaloid, Wagner's was performed, where initiallysolvent free extract 50 mgwas mixed with few ml ofdilute hydrochloric acid and then filtered, the filtrate is used for testing the presence of alkaloids. To a few ml of filtrate, a few drops of Wagner's reagent were added by the side of the test tube. A reddish brown precipitate indicates the presence of alkaloids (6).

Test for Oil: A small quantity of extract was pressed between two filter papers; oil stain on the paper indicates the presence of fixed oil [7].

Test for Tannins:10 ml aqueous extract was mixed with 0.1% of Ferric chloride.The positive results for the presence of tannin to form green colour to blue black. (8).

Test for Flavonoids: 2ml of the each extract was added to 2ml water and 5ml of 20% NaOH. The yellow colouration formed to show the positive results of flavonoids. (9)

Test for saponins: About 2g of the powdered sample was boiled in 20ml of distilled water bath and filtered. The 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a suitable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, and then the formation of emulsion was observed. (3)

Test for Coumarins: 3 ml of 10% NaOH was added to 2ml of aqueous extract. Formation of yellow colour indicated the presence of Coumarins. (5)

Salkowski's Test: It is the test for terpenoids. 5ml of each extract was mixed in 2 ml of chloroform and 3ml of concentrated H2SO4 was carefully added to a form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoid. (8)

Elemental analysis: Samples are prepared as by taking2gmsofsamples,andadd10 ml of conc. nitric acid. Transfer them in to crucibles and keep them inside furnace at 200degrees.Take out the ash formed in the crucibles. Dilute them with water and filter the supernatant. Standards are prepared as per, and readings are noted with the help of Atomic absorption spectroscopy [6].

Test for carbohydrates: The extracts were treated with 5.0 ml of Fehling's solution and kept in boiling water bath. The formation of yellow or red colour precipitate indicates the presence of reducing sugars. (3)

Estimation of crude fiber: Extract 2 g of ground material of seed with ether or petroleum ether to remove fat (Initial boiling temperature 35-38° C and final temperature 52° C). If fat content is below 1%, extraction may be omitted. After extraction with ether, boil 2 g of dried material with 200 ml of sulphuric acid for 30 min with bumping chips. Filter through muslin and wash with boiling water, until washings are no longer acidic. Boil with 200 ml of sodium hydroxide solution for 30 min. Filter through muslin cloth again and wash with 25 ml of boiling 1.25% H_2SO_4, three 50 ml portions of water and 25 ml alcohol. Remove the residue and transfer to ashing dish. Dry the residue for 2 h at 130 ± 2° C. Cool the dish in desiccators. Ignite for 30 min at 600 ± 15° C. Cool in desiccators [6].

Glycosides: 50 mg of extract was mixed with few ml of conc. hydrochloric acid for 2 hours on water bath and then filtered; the filtrate was used for testing the presence of glycosides by legal's test. 0.5 ml of filtrate was dissolved in pyridine, and then sodium nitro prusside solution was added and made alkaline using 10% sodium hydroxide. Pink color indicates the presence of glycosides [6].

Total protein: The total protein content in the seeds is estimated by Lowry's method. Different dilutions of BSA solutions are prepared by mixing stock BSA solution (1mg/ml) and water in the test tube, as given in the table.The final volume in each of the test tubes is 5 ml. The BSA range is 0.05-1 mg/ ml. From these different dilutions, pipette out 0.2 ml protein solution to different test tubes and add 2 ml of alkaline copper sulphate reagent (analytical reagent). Mix the solutions well. This solution is incubated at room temperature for 10 mins.Then add 0.2 ml of reagent Folin Ciocalteau solution (reagent solutions) to each tube, and incubate for 30 min. Zero the colorimeter with blank and take the optical density (measure the absorbance) at 660 nm. Plot the absorbance against protein concentration to get a standard calibration curve.Check the absorbance of unknown sample, and determine the concentration of the unknown sample using the standard curve plotted above [10)

Totalfat: The total fat content was determined by extraction of 2.0-2.5 gof dry ground sample for 12 h in a Soxhlet with petroleum ether, and removed the solvent by rotary evaporator, then dried the sample in hot air oven at 100° C for about 1 h to allow the ether evaporate (11-14).

Test for Pigments:1 ml of extract was extracted with10 ml of chloroform in a test tube with vigorous shaking and then 85% sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids. (9)

Test for combined anthraquinones

1ml of extract was boiled with 2ml of 10% Hcl for 5 mins. The cooled filtrate was extracted with equal volume of ch-loroform and the chloroform layer was transferred to dry clean test tube. 10 % ammonia solution was added in the chloroform layer, shaken well and allowed to separate.These parated layer was observed for colour change. Pink colour is the positive result for combined anthraquinones. (9).

RESULTANDDISCUSSION

Reducing sugars were tested against Fehling solution showed positive indication of yellow(or) red precipitate appeared in Water and Methanol. Phenol appeared in Water extract where as absent in Methanol, they showed blue and black colouration.Steroids were tested against Chloroform, Positive results appeared green to yellow colouration both inWater & Methanol.Wagner's test was performed to test for Alkaloids appeared as reddish brown precipitate in both in water and methanol extracts. Salkowski's test was done to identify terpenoids in the seeds, reddish brown colour indicates the presence of Alkaloids in water and methanol.They were the important component in the plant which acts as astringent (15). Flavanoids are major group of phytochemicals present in the plants, they were showed yellow ccolour in both water and methanol. Coumarins are aromatic compounds, widely used to enhance aroma.Thy are naturally found in plants in a crystalline form, yellow colour formation promised the coumarins presence in the seed, only appeared in water extracts. A clear emulsion was formed in water extract where as absent in methanolic extract.Glycosides present in both extracts showed pink colouration.Carotenoids showed blue colour interface in both extractions. Alkaloids, Fibre, Glycosides and Protein were tested by using standard official methods of AOAC methods.Only fat content was tested by used Petroleum ether.

Phenols, flavonoids tannins and coumarins are responsible for chemo preventive properties like anti-oxidant, anti-carcinogenic and anti-inflammatory effects (16).The Alocholic extracts of Lablab purpureus leaves and seeds reduced blood glucose (17), where as methanolic extracts of some doses controls blood glucose ranges as well as body weight in animals (18).Aqueous extract of Dolichos bean canincrease significant change in heamoglobin percentage (19) which shows anti-inflammatory (20) analgesic effect, antioxidant activity (21) cytotoxic activity (22) Hypolipidemic effect (23) Anti- microbial effect (24) insecticidal effect (25) Hepatoprotective effect (26) Antilithiatic activity (27) along with this side effects and contradictions also present.

SUMMARY&CONCLUSION:

The above study discussed the chemical constituents and pharmacological effects of Lablab purpureus have the wider an geof medicinal properties have been used for many medical applications.

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