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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS OF SEED EXTRACTS *Macrotyloma uniflorum* (HORSE GRAM)

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ABSTRACT

Macrtotyloma uniflorum (Horse gram) is one of the lesser known beans also called as "Poor man's pulse". In this study, the phytochemicals present in the horse gram were analysed with various solvent seed extracts. The solvent ranges from no polarity (hexane) to high polarity (water) were used for extraction. The qualitative and quantitative assessment of phytochemicals of the horsegram plant seed shows the medicinal value of the plant.

INTRODUCTION

In the plant kingdom, every plant has the potential to produce primary and secondary metabolites which are bioactive compounds. These compounds play a vital role as plant medicine in curing many diseases. It includes flavanoids, terpenoids, glycosides, tannins, steroids, and saponins etc. Bioactive compounds from the plant sources have the broad spectrum of Anti- bacterial, Anti-fungal and Anti-oxidant activity. Dolichins from the Horsegram plant posses Anti HIV properties (Rufus et al., 2013). It helps the human race from various diseases and helps to lead a healthier life. Bioactive compounds are the source for many drug designing processes. So it is very important to explore the phytochemicals of such plants to help the mankind. *Macrotyloma uniflorum* is leguminous plant belongs to the family: Fabaceae (alt. Leguminosae). They are twining, annuals with tomentose cylindrical stems. Pod shortly stipitate, slightly curves, smooth or tomentose. Seeds ovoid, pale fown, light red, brown, or black sometimes with faint mottles or with small, scattered black spots (Blumenthal et al., 1989). It is an excellent source of iron and molybdenum and vegetable proteins (Kadam *et al.*, 1985, nigwekar *et al.*, 1991). It has the greatest potential for the utilization of nutraceuticals, forage and food for malnourished and drought prone areas of the world (Morris, 2008). Herbal medicine is the part and parcel of the much needed health care in most of the developing countries (Kawsar *et al.*, 2008). In the present study, the detailed phytochemicals analysis of horse gram plant seeds were discussed.

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MATERIALS AND METHODS

Paiyur variety seeds were purchased from the TNAU, Paiyur. 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 hexane, diethyl ether, butanol, chloroform, methanol, acetone, and water were subjected to soxhlet extraction. For every solvent 250mg of seed powder was used against 150 ml of solvent. Fractions were collected after some rounds of successful extraction in the soxhlet apparatus.

Phytochemical tests

All the seven extracts were tested for phytochemicals using standard protocols and results were listed out in the table. Table 1 were done to find out the bioactive ingredient such as Terpenoids, Tannins, Steroids, Leucoanthocyanins, coumarins, Flavanoids, phlobatannins, anthraquinones, saponins, cardioglucosides, proteins and carbohydrates.

Salkowski's Test

It is the test for terpenoids. 5ml of each extract was mixed in 2 ml of chloroform and 3ml of concentrated H_2SO_4 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. (Doss *et al.*, 2009).

Table 1. Phytochemical analysis of various solvent extracts of *Macrotyloma uniflorum* seeds

TEST	Hexane	Diethyl ether	butanol	Chloro Form	Methanol	Acetone	Water
Salkowski	+	+	+	+	+	+	+
Tannins	NA	NA	NA	NA	NA	NA	+
Anthocyanins	_	_	_	_	_	_	_
Leucoanthocyanins	NA	NA	NA	NA	NA	NA	+
Coumarins	NA	NA	NA	NA	NA	NA	+
Flavonoids	_	+	+	_	+	+	+
Phlobatannins	NA	NA	NA	NA	NA	NA	+
Emodins	_	_	_	_	_	_	_
Steroids	_	_	+	_	+	+	+
cardioglucoside	_	+	+	+	+	_	+
Phenols	_	_	_	+	_	+	+
Carotenoids	_	_	_	_	_	_	_
Combined Anthraquinone	_	_	_	_	_	_	_
Free Anthraquinone	_	_	_	_	_	_	_
Reducing Sugars	_	_	+	_	+	+	+
Carbohydrates	+	_	+	+	_	+	+
Saponins	_	_	_	_	_	-	+

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. (Doss *et al.,* 2009).

Test for Anthocyanins

2ml of aqueous extract was added to 2ml of 2N HCl and ammonia. The appearance of pink- red turns blue violet indicated the presence of anthocyanins. (savithiramma *et al.,* 2011)

Test for Leucoanthocyanins

5ml of the aqueous extract was added to 5 ml isoamyl alcohol. Upper layer appeared red in colour indicated the positive results for leucoanthocyanins. (savithiramma *et al.,* 2011).

Test for Coumarins

3 ml of 10% NaOH was added to 2ml of aqueous extract. Formation of yellow colour indicated the presence of Coumarins. (savithiramma *et al.,* 2011)

Test for Flavonoids

2 ml of the each extract was added to 2 ml water and 5 ml of 20% NaOH. The yellow colouration formed to show the positive results of flavonoids. (Maobe *et al.,* 2013)

Test of Phlobatannins

Deposition of red precipitate of when an aqueous extract of seed was mixed and boiled with 1% aqueous HCl was taken as appositive evidence for phlobatannins.(Ajayi *et al.,* 2011)

Test for Emodins

2ml of NH_4OH and 3ml of Benzene were added to the extract. Appearance of red colour indicated the presence of emodins. (savithiramma *et al.,* 2011)

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. (savithiramma *et al.,* 2011)

Test for Cardio glycosides (Keller- kiliani test)

1ml of glacial acetic acid was added to the 1 ml of extract containing one drop of ferric chloride. Subjected with conc sulphuric acid formed ring ranges from green to brown according to the type of cardio glycosides presented. (Maobe *et al.,* 2013)

Test for phenols

2% $FeCl_3$ was added to the 1ml of sample formed blue black colouration. This showed positive result for phenols. (yadhav *et al.,* 2011)

Test for carotenoids

1ml of extract was extracted with 10ml 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. (Maobe *et al.,* 2013)

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 chloroform 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. The separated layer was observed for colour change. Pink colour is the positive result for combined anthraquinones. (Maobe *et al.,* 2013)

Test for free anthraquinones

5 ml of chloroform was added to the 2ml of sample shaken well and filtered. The filtrate was then mixed with equal amount of 10% ammonia. The bright pink colour indicated the presence of free anthraquinones. (Maobe *et al.,* 2013)

Test for Reducing sugars

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. (Thenmozhi *et al.,* 2011)

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. (Thenmozhi *et al.,* 2011)

Test for saponins

About 2 g of the powdered sample was boiled in 20 ml 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. (Thenmozhi *et al.,* 2011)

Analysis of fluorescence characters

Various chemical compounds were mixed with crude powder and viewed under white light and UV light. The observations were listed in the Table 2.

Table 2. Analysis of Fluorescence characters of seed powder

Sl.no	Composition + SP	White Light	UV light
1.	10% NaOH	Fluorescence green	No change
2.	20% NaOH	Yellow	Fluorescence green
3.	Conc. Sulphuric acid	Brown	Browinsh black
4.	Acetic Acid	Light yellow	green
5.	KNO_3	Yellow	No change
6.	NaOH in Ethanol	Fluorescence green	No change
7.	$FeCl_3$	Orange	Brown
8.	Nitric Acid	Yellow fluorescence	Yellow fluorescence
9.	HCl	Orange	Brown
10.	Acetone	pink	pink

SP- seed powder

RESULTS AND DICUSSION

Salkowski's test is for terpenoids identification, for all the seven extracts brown ring formed in the interface between chloroform and sulphuric acid. This is the postivie indication of terpenoids in the seed extracts. Tannins were the important component in the plant phytochemicals which act as an astringent. A green colour formation was there due the addition of few drops of 1% ferric chloride, confirmed tannin presence in the seeds of Horse gram. Red colour formed when isoamyl alcohol was added to the extract showed the presence of leucoanthocyanins. Leucoanthicyanins are the intermediate chemical compound in the anthocyanins synthesis. Whereas anthocyanin test showed negative results. Coumarins are aromatic compounds , widely used to enhance the aroma. They are naturally found in plant in a crystalline form. Yellow colour formation promised the coumarins presence in the seeds when sodium hydroxide was added to aqueous extract. Flavonoids are major group of phytochemicals present in the plants. Hexane and chloroform showed negative results where as other extracts like diethyl ether, acetone, methanol, water and butanol extracts formed yellow colour which showed the positive results for flavonoids. Aqueous extract of seeds of *Macrotyloma uniflorum* was positive for phlobatannins and there were no presence of emodins, in all the seven extracts. Upper layer turns no change but sulphuric acid layer turned as yellow green ring in the hexane, chloroform, and diethylether extracts in the steroids test. For, acetone, methanol, water and butanol the upper layer turned red and sulphuric acid layer turned brown which is the postitive indication of the presence of steroids. The cardiac glycosides test is otherwise called as Keller – kiliani test to find out the type of the sugar present in the extracts. Diethyl ether, methanol, water butanol extracts showed brown ring which represents the presence of " deoxy sugar of cardenolids. Carotenoids, combined anthraquinone and free anthraquinones were not to be found in the seeds of horsegram.

Reducing sugars were tested against fehling's solution, precipitate formed in butanol, methanol, acetone and water, positive indication. The remaining extracts were negative for reducing sugars. Saponin test was positive for aqueous extract. Fluorescence studies play a vital role in the case of various compounds present in a single plant which give fluorescence when mixed with chemicals viewed under UV. The results were listed in the Table 2. The fluorescent colour is specific for each compound (Sridhar s *et al.*,1999). 10% NaOH was mixed with seed powder showed green fluorescence when viewed under white light and UV light. Whereas 20% NaOH showed yellow under white light and fluorescence green under UV light. Concentrated sulphuric acid showed Brown and Brownish black under white and UV light respectively. Seed powder in acetic acid was in pale yellow under white light but green in uv light. There was no change in KNO_3 with seed powder under UV light but showed yellow colour under white light. NaOH Ethanol showed fluroscence green with seed powder under white light, no change after the exposure of UV. $FeCl_3$ showed orange under white light but brown under UV light. Nitric Acid showed yellow fluorescence under white light and UV light. Hcl showed orange colour under white light where as brown in UV light. Pink colour was for acetone mixed with seed powder both white and UV light. In the present study of photochemical analysis of seed extracts of *Macrotyloma uniflorum* evidenced the various phyto-chemicals and present in the plant which are widely used as a drug. It may lead to further identification of therapeutically important compounds for the drug designing processes.

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