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Phytochemical investigation on *Jatropha curcas* seed cake

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Abstract

Since ancient times plants have been used as source of therapeutic agents. Plants are playing a significant role in the indigenous system of medicine to combat diseases. Plants are the richest sources of bioactive organic chemicals on earth. They are the store house of secondary metabolites such as alkaloids, terpenoids, steroids and flavonoids etc. The traditional medicine involves the use of different plant extracts or bioactive chemicals. This type study provides the health application at affordable cost. This such as ethanomedicine keenly represents one of the best avenues in searching new economic plants for medicine.In keeping this view in mind the present investigation is carried out in *Jatropha curcas* seed collected in June 2008 from Banthra Research station Lucknow, India. The results suggest that the phytochemicals properties of the seed for curing various ailments.

Keywords: *Jatropha curcas* , n-hexane, Ethyl acetate, Traditional medicine, Phytochemical.

Introduction

Phyto" is the greekword for plant. There are many "families" of phytochemicals and them helps the human body is variety of way. Phytochemicals may protect human from a host of disease. Phytochemicals are non- nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemical to protect itself but recent research demonstrates that many phytochemicals can protect humans against disease. These are many phytochemicals in fruit and shrubs and each work differently.

Jatropha curcas (physic nut, purging nut), a tropical plant belonging to the family of *Euphorbiaceae* is cultivated mainly as a hedge in many Latin American, Asian and African countries. They are use as a traditional medicine. These traditional medicines involve the use of different plant extracts or the bioactive constituent. *Jatropha curcas* seed cake (JCSC) contains various toxins and is therefore not usable as fodder[1]. However the raw seed cake can be valuable as organic nutrient source as it contains more nutrients seed cake is useful as fertilizer. The presences of the aforementioned bio-degradable toxins, mainly phorbol esters, make the fertilizers cakes simultaneously serve as bio pesticide / insecticide and molluscicide[1] and it is advisable to check the absence phorbol ester in the crop grown on *Jatropha curcas* seed cake fertilized land, certainly crops for human consumption this type of study provide health as well as fertilizer application at affordable cast this study such as ethno medicine keenly represents one of the best avenues in searching new economic plants for medicine. In keeping this view in mind the present investigation is carried out in *Jatropha curcas* seed cake (JCSC) and their result suggest that the phytochemicals properties of the seed for curing various alignment.

Material and methods

Plant materials

Jatropha curcas seed collected in June 2008 from Banthra Research station Lucknow. The plant material was identified in Ethanobotany section at the National Botanical Research Institute (CSIR), Lucknow, India.

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Preparation of extracts

Jatropha curcas seed cake is grinding then extracts with n-Hexane to removes lipids. It was then filtered and the filtrate was discarded. The residue was successively extracted with different solvents as such ethyl acetate, n-Butanol, Acetone: water and methanol material will concentrate in reduced pressure at below 50^0C on rotary evaporator for phytochemical screening.

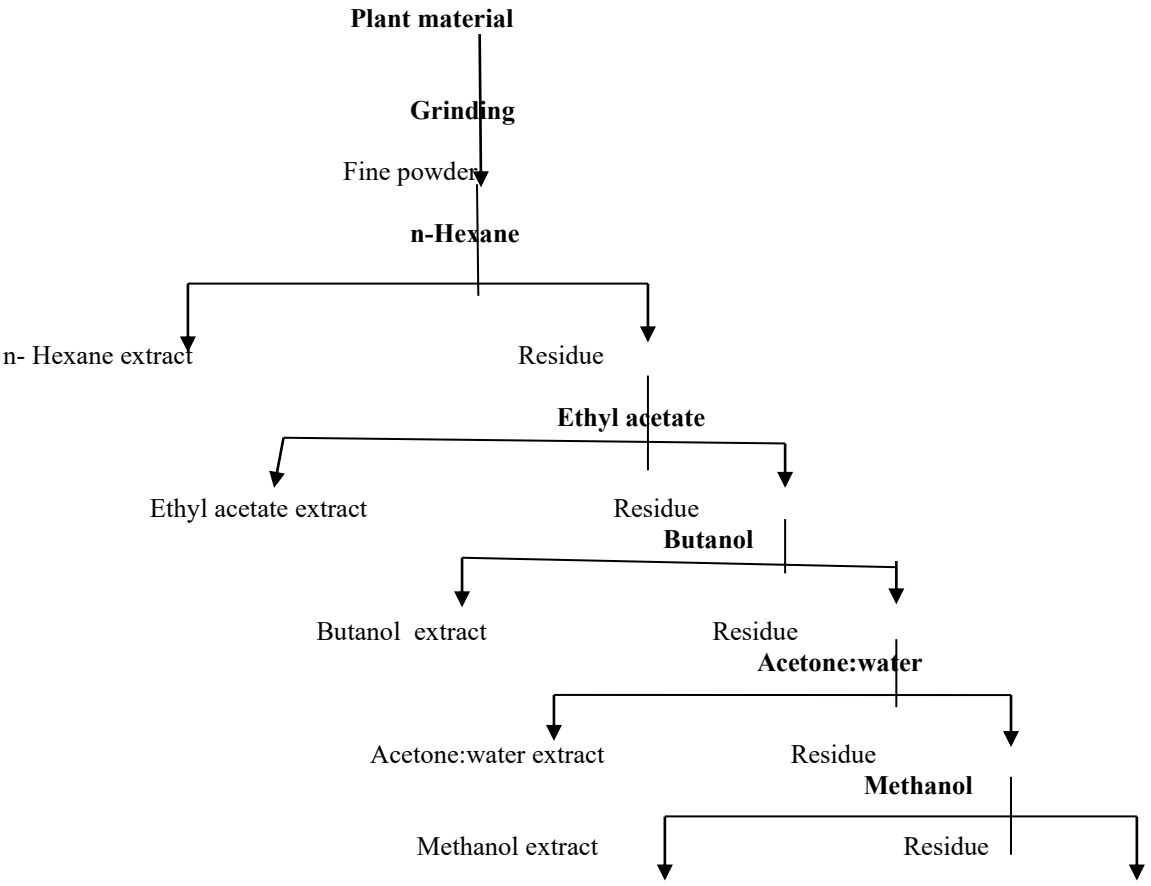

Figure 1: Systematic Extraction Process Secondary Metabolites from *Jatropha curcas* seed cake

Physiochemical parameter

Oil Content

The weight of oil extracted from taken of seeds powders was measured to determine the lipid content.

$$\text{Oil\%} = \frac{\text{Wt. of oil}}{\text{Wt. of material taken}} \times 100$$

Result was expressed as the percentage of oil in the dry matter of seed powders.

Acid value, % FFA

Acid value of seed oil was determined percentage free fatty acids (FFAs) were calculated using oleic acid as a factor. Taken the sample 1gm (moisture free) and added 20 ml alcohols previously neutralized to phenolphthalein and titrate with 0.1 N KOH solution until the solution remains faintly pink and volume.

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$$\text{Acid value} = \frac{56.1 \times V \times N}{\text{Mass of material}}$$

Where, V=Titre volume of KOH, N=Normality of KOH

Iodine value

Iodine value of seed oil was determined unsaturation of an oil or fat. Taken 0.5 gm oil sample in 500ml conical flask and added 20 ml chloroform and dissolved completely and added 25ml Hanus iodine solution mixed well titrate against standard 0.1 N sodium thiosulphate solution and using starch as indicator with vigorous shaking to extract the iodine from the chloroform layer and conduct blank similarly in absence of oil .

$$\text{Iodine number} = \frac{12.69 \times (\text{Blank} - \text{Titre}) \times N}{\text{Wt. of material}}$$

Where, N=Normality of $Na_2S_2O_3.5H_2O$

Saponification value

Take 1gm sample and added 25gm 0.5N alcoholic KOH and reflux it on water bath for 1hour. Cool it and added 20 ml distill water and few drops of phenolphthalein as indicator mixed then titrate against standard 0.5N HCl until the pink colour disappears and treat the blank similarly in the absence of oil .

$$\text{Saponification} = \frac{56.1 \times N \times (\text{Blank-Titre})}{\text{Wt. of oil}}$$

Density

The density of the samples was determined at 25 °C, take weight of 1ml sample.

Preliminary phytochemical screening : One gram of Ethyl acetate, Butanol, Acetone:water (7:3) and Methanol extract of JCSC were dissolved in 100 ml its own mother solvents to obtain a stock of concentrate 1%(V/V) .The extract obtained subjected to preliminary phytochemicals screening following the methodology of [2-3]

Screening procedure

Test for alkaloids: To 5ml stock extract and added 2ml HCL make acidic medium and then added 1ml of dragendroff's reagent then orange or red colour precipitate obtained. Immediately indicated presence of alkaloids.

Test of amino acid: Add 40% NaOH solution to 2 ml stock extract in test tube. Now add one drop of 1% $CuSO_4$solution. Appearance of blue colour shows the presence of amino acids.

Test for anthraquinones: Hydrochloric acid (2M) was added to the sample and the mixture was heated on a hot water bath for 15 minutes, then cooled and filtered. The filtrate was extracted with chloroform. The chloroform layer was separated and shaken with 10% potassium hydroxide solution. The upper aqueous layer becomes pink-red shows the presence of anthraquinones.

Test of flavonoids: To one ml extract was add dilute NaOH (1N) then instance yellow colour in plant extract, which comes colourless on addition of few drop acid (10%H_2SO_4) indicate presences of flavonoids.

Test of glycosides: 1ml extract hydrolysed with HCl for few hours on water bath and cool at room temperature add 1ml pyridine and few drops sodium nitropruside solution and made alkaline with NaOH solution appears pinks to red colour indicate presence of glycosides.

Test of phytosterol: The extract was refluxed with solution of alkali KOH till complete Saponification takes place. The mixture was diluted and extracted with ether .The ether layer was evaporated and the residue was tested for presence of phytosterol. The residue was dissolved in few drops of dilute acidic acid, 3ml acetic anhydride was added followed by few drops of concentrate H_2SO_4 appearance bluish green colour, indicate presence of phytosterol.

Test for sponins: To extract diluted with 20 ml of distilled water and agitated in a graduated cylinder for 15 minutes then formation of layer (1cm) foam shown presence of sponines.

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Test of steroids: To 1ml extract was dissolved in 10 ml Chloroform and 10 ml concentrated sulphuric acid by side of test tube then formation of upper layer turned red colour and sulphuric acid layer shows yellow with green fluoresces, indicates presence of steroids .
Test for tannins: To 5ml extract was add few drops 1% lead acetate appearance yellow precipitate, indicates presence of tannins.
Test for triterpenoids: Taken 1mg extract was dissolved 1ml chloroform, 1ml acetic anhydrides and 2ml concentrated sulphuric acid appears reddish violet colours indicates the presence of triterpenoids .

Physical parameter of *Jatropha curcas* seed cake oil

Plant material *Jatropha curcas* seed cake [JCSC] extracted in different solvent i.e. n-Hexane, Ethyl acetate and Methanol. n-Hexane used for removal of oil from plant material and collected data from the study of physical parameter of oil i.e. percentage of oil, colour, pH, Refractive index, density, Acid value, Iodine value and Saponification value. The physical parameters of seeds cake are showed in Table 1.

Results and Conclusion

Physical parameter (Table 1) of *Jatropha curcas* seed cake was oil content, 7.45%; colour, golden yellow; pH, 6; refractive index 1.4556nD (30.9°C);density(mg/ml), 0.8728; Acid value (mg KOH/gm,4.21); iodine value,(111.5); Saponification value(mg KOH/gm), 169.9. Acid value of JCSC was 4.21mgKOH/gm. The seed cake will contain 3.71mgKOH/gm. This determined free fatty acid in JCSC oil. This value indicates that the oil quality is dependent on the interaction of environment and genetics as seed size, seed weight and oil content[4]. The iodine value of *Jatropha* oil was determined at 111.5g I_2/100g; higher iodine value indicated that higher unsaturation of fats and oils. The limitation of unsaturated fatty acids is necessary due to the fact that heating higher unsaturated fatty acids results in polymerization of glycerides. This can lead to the formation of deposits or to deterioration of the lubricating[5]. The iodine values of JCSC place them in the semi-drying oil group. High iodine value of *Jatropha* are caused by high content of unsaturation fatty acid such as oleic acid and linoleic acid The usual method of assessment hydro peroxides (primary oxidation products) is by determination of peroxide value[6] . The high iodine value and oxidative stability shows that the seed oil upholds the good qualities of semidrying oil purposes[7]. Saponification value of the studied oil were 169.9mg.KOH/gm. High Saponification value indicated that oils are normal triglycerides and very useful in production of liquid soap and shampoo industries.
Jatropha seeds cake extract such as n-Hexane, Ethyl acetate and Methanol contains different secondary metabolites (phytochemicals) with biological activity that can be of voluble therapeutic index. Different phytochemicals (Table 2) have been found in polar and non polar solvent to passes a wide range activities, which may help in protection against a number of several toxic or anti- nutritional compounds, for example Alkaloid protect against chromic disease[8-11]. Sponins protect against hypercholesterolemia and antibiotic properties. The terpenoids have been shown to disease blood sugar level in animal studies[8], Steroids and Triterpenoids shows the analgesic properties. The Steroids and Sponins were responsible for central nervous system activities[12]. Phytochemicals screening of the n-Hexane, Ethyl acetate and Methanol extract of *Jatropha* seeds cake used to study of the crude extract contained alkaloids, amino acid, anthraquinone, flavonoids, glycosides, phytosterol , sponines, steroids, tannins and triterpenoids and also have various medicinal values such as anti-inflammatory, anti-diabetic and analgesic activities and for central nervous system activity. Further studies are in progress in our laboratory to isolate the active component.

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Table 1: **Physical parameter of *Jatropha curcas* seed cake oil**

S./No.	Parameters	Results obtained
1.	Colour	Golden yellow
2.	pH	6
3.	Refractive index	1.4556nD(30.9oC)
4.	Oil %	7.45
5.	Acid value(mg KOH/gm)	4.21
6.	Iodine value(mg iodine/gm)	111.5
7.	Saponification value(mg KOH/gm)	169.9

Table 2: Analysis of phytochemicals in following extract of *Jatropha curcas* seeds cake.

Test	n-Hexane extract	Ethyl acetate extract	Butanol extract	Acetone: water extract	Methanol extract
Alkaloids	-ve	+ve	-ve	-ve	+ve
Amino acid	-ve	-ve	+ve	+ve	-ve
Anthraquinones	-ve	-ve	-ve	-ve	-ve
Flavonoids	+ve	+ve	-ve	-ve	-ve
Glycosides	-ve	-ve	-ve	-ve	+ve
Phytosterol	-ve	-ve	+ve	-ve	-ve
Sponins	-ve	-ve	+ve	+ve	+ve
Steroids	-ve	-ve	+ve	-ve	-ve
Tannins	-ve	+ve	+ve	+ve	+ve
Triterpenoids	+ve	+ve	-ve	-ve	+ve

+ve = Presence; -ve = Absence

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