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EVALUATION OF ANALGESIC OF PUMPKIN SEED'S (CURCUMA PEPO) EXTRACT IN EXPERIMENT ANIMAL

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ABSTRACT

The use of herbal remedies individually or in combination with standard medicines has been used in various medical treatises for the cure of different diseases. Pumpkin is one of the well-known edible plants and has substantial medicinal properties due to the presence of unique natural edible substances. It contains several Phyto-constituents belonging to the categories of alkaloids, flavonoids, and palmitic, oleic and linoleic acids. Various important medicinal properties including anti-diabetic, antioxidant, anti-carcinogenic, anti-inflammatory and others have been well documented. The purpose of the present article is to discuss various medicinal and biological potentials of pumpkin that can impart further research developments with this plant for human health benefits. Pumpkin: Herbal medicine: Anti-diabetic properties: Antioxidants: Anti-carcinogens: Phytochemicals.

KEYWORDS: Herbal medicine, Pumpkin, Curcuma Pepo and Analgesic Activity.

INTRODUCTION

Pumpkin are cultivar of most the commonly used Cucurbita Pepo, that's round and found with smooth and slightly ribbed skin with deep yellow to orange color. The thickened shell contains seed and pulp. Some very large cultivars of these squash are also derived with the similar appearance such as Cucurbita Maxima, some specific cultivars of winter squash are also derived, from others species too, including Cucurbitaargyrosperma and cucurbitamoschata are also commonly called as "PUMPKINS" in some countries.

The term Pumpkin is generally referring to the broader category named winter squash or simply a Squash. Native North American pumpkins are grown widely for Commercial use

only and are being used in both food as well as in recreations. Pumpkin's pie, is a traditional part of giving thanks in Canada and America by giving meals with the squash, although commercially grown Pumpkins Puree and Pumpkin Pies are usually made of different kinds of winter squashes than the Pumpkins are frequently being carved as a jack-o'-lantern for large decorations around hallow ween.

Fig.1.1 Pumpkin Fruit.

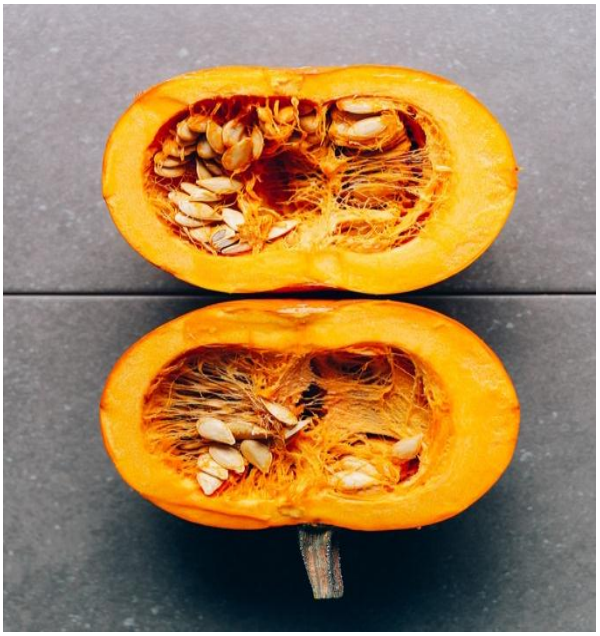

Fig1.2 Pumpkin seeds.

PUMPKIN (Cucurbita sp.) is known since the ancient times till today also. Today, pumpkins are widely cultivated and being used for food and decorative purposes. Pumpkin seeds contribute significantly to the Nutrition of living populations in various parts of the world. The main nutritional relevant components of Pumpkin seeds are Proteins (30-51) and Oils (up to 40%), also rich in Carbohydrates (10%) and micro elements like nutrients (up to 4-7%). Differences in the chemical composition of Pumpkin seeds, b/w Cucurbita species and cultivars from the different parts of the area in world might be related to growth and fertilization conditions and the harvesting time too.

Classification of Pumpkin Seeds

Kingdom - Plantes- Plants, Plants, Vegital,

Sub- kingdom - Viridiplantae

Infra- kingdom - Streptophyta - Land planet

Super division - Embryophyta

Division - Spermatophytina (Spermatophyte's plant).

Class. - Mangnoliopsida

Super order - Rosa are

Family - Cucurbita L- gourd

Species - Cucurbita maxima duchesne - winter squash

Description of Plant (Pumpkin plant)

Pumpkin plants are having large leaves, Sprawling coiled veins, hair like curly tendrils. The leaves are simple, wide and shallow to deeply lobed. The roots are in the surface, Stream might be square, flowers are bright yellowish in colour and the fruits are more fibrous and lesser sweet in taste than winter squashes. Seeds vary in size and shape depending upon variety any breed of Pumpkin, it will have a natural protective skin which we call the hull. The Pumpkin's plant has been growing since the Ancients history and time of the mankind. The animal species are probably natives of Asia but now got acclimatized to temperate and climate changes.

MORPHOLOGICAL STUDIES

Physical Characteristics

Cucurbita's are herbaceous and perennials with extensively branched roots and having moist vines. The plants are grown either prostrates along the ground or climbs using tendrils, they rarely grown as trees, shrubs or bushes. The tendrils can be grown branched or simple and generated at the Petiole base. These are usually four arched filamented coils with an adhesive texture, leaves can be ranged from simple to palmately compound. Those leaves that develop later are more deeply lobed and extra floral nectarines are often found. Flowers are unisexual, so the plants are male or female, rarely hermaphroditic.

Chemical Constitution of Cucurbita Maxima

Pumpkin (C. maxima) seeds are a very good source of vitamins, mostly the vitamin B along with C, D, E, K. Beside zinc, magnesium manganese, phosphorus, phytosterols. It contains chemical constitutions such as 24 beta ethylene 5 alpha, cholesterols 72,225, tries- e beta ol 24 beta ethylene 5 alpha cholesterols, 7,2,5 dien 3 bet ol, arena sterol spina sterols 2, 4 methylene lathi sterol and 25(27) dehydrofungisterol. C-NMR of condrillsterols studied and isolation of new carotenoids cucurbita xanthin (A&B) and their structure elucidation done. The seeds collected from Pant nagar yield and Oil (39.5%) having the following physical chemical constituents, the fatty acids composition of Oil is as follows myristic,

palmitics, stearin, oilic, and linoleic acid 30.3%. The alcoholic and ethereal extracts of seeds were found to be active against the nematode facia.

Pumpkin's seed oil

The oil is main exported commodity of Austria and Slovenia. Crushed/pressed roasted pumpkin hull less seeds are used for making this oil, from a local variety of Pumpkins, the pumpkin oil (Cucurbita pepo, var. Styriaca, also known as var. Oleifera). It has been produced and used in styria's southern parts at least since the 18th century. The Oil colour depends on the type of pumpkin used, its colour ranges from light to very dark red.

Fig. 1.11: Pumpkin Seed Oil

Colorimetric Method

In physical and analytical chemistry, colorimetry is "used to determine the concentration of coloured compounds in solution". The device used to test the concentration of the solutions by measuring its absorbance for a specific wavelength of light is colorimeter. Different solutions are prepared for colorimeter, a controlled reference solution is prepared with known concentration with the help of visual colorimeter for example Dubos colorimeter. The filtered light transmission through the prepared solutions is compared for the visual match and the wave length of the light passed through them can be varied.

MATERIALS AND METHODOLOGY

List of Material

Instruments	Company	Model
UV-Visible Spectroephotometer	Systronics	Model no. 2203
Digital Balance	Citizen	Model Cy220,max 220g,
Hot Air Oven	Science Tech	Line 220 VAC
Heating Mental	Science Tech	SIZE 250 ml
PH meter	Max	ME963P
UV Chamber	Systronics	2203R
Hot Plate	S4S Eddy hot plate	NU-207
Pentazocine	Cipla	-
PEG	Sun Pharma	-

Soxhlet Extraction Procedure

The purchased seed material from market is screened for any impurities and dried in an oven. Dried seeds were grinding to coarse powder using an electric grinder, sheaved (80 mesh) and stored in air tight jars until use. The Dried powder seeds were extracted by Soxhlet apparatus using Hexane, chloroform, methanol, ethanol, and water successively. Each time before employing the solvent of higher polarity Marc was dried. Each extract was then concentrated using rotary vacuum evaporator at 40-50 degree Celsius under vacuum and dried residue was collected and refrigerated for further experimentation.

Preliminary Phytochemical Screening

The Hexane, Chloroform, Methanol, Ethanol and Water extracts of Cucurbita maxima will be screened for following phytoconstituents class. The method for the preliminary phytochemical screening was carried away as per Pharmacognosy C. K. kokate.

Test for the presence of Alkaloids

Evaporate the ethanolic extract saperetly, add the dilute HCl and shake well and now filter by using a filter perform the followings test. Dragendorff, test: Dragendorff,s reagent (DR) This reagent is used to detect the presence of alkaloids and heterocyclic nitrogen compounds. Solution A Dissolves 0.85gm of basic bismuth nitrate in 10 ml glacial acetic acid 40 ml of H2O under heating.

Solution B is dissolved to 8gm of KI in 30 ml H2O

Both the Soln. A and B were mixed in the proportion 1:1 and is used for spraying.

Mayer' s test

Mayer' s Reagent

Dissolve 1.36gm of mercuric chlorides in 60 ml distilled H2O; add it to the solution of 5gm of KI in 20ml of distilled H2O make the volume up the 100ml add few drops Mayer's reagent in 2-3 ml of filtrate. Cream colour precipitates formation.

Wagner, s Reagent

Dissolve1.27gm of iodine and 2gm of Poterium iodine in 5ml of H2O and make up the volume to 100 ml with distilled H2O. To 2-3ml filtrate with few drops Wagner's reagent. Yellow colour precipitates formation.

GLYCOSIDE'S PRESENCE TEST

Cardiac glycoside's presence test

The test includes Baljet, s test, Legal test, Keller-killiani test a Liebermann' s test. The conformation was done by the colour change after the reaction with the various reagents.

Baljet test: Yellow to orange colour with sodium picrate.

Legal's test; Add 1ml pyridine and sodium nitroprusside each for colour change from pink to red.

Killarkilani Test 2 ml of extracts added to Glacial acetic acid one drop of 5% FeCl3 and concentrates H2S04. At the junction of two liquid layers radish brown colour appears and bluish coloured upper layer.

Liebermann- Burchard Resection: 3ml extract and acetic hydride each are mixed. Then Heat and cool. Few drops conc. H2SO4 added gives blue colour.

Coumarin Glycoside test

Blue and green fluorescence colour appeared Hawn alcoholic extract is made alkaline. Other test is with dilute NAOH which gives yellowish green fluorescence in UV light.

Test for Flavonoids

One of the main tests for the presence of flavenoid is the Shinoda test with emploid the used 5ml of 95% ethanol with few drops of concentrated HCL and 0.5g of magnesium turnings. The result gives a pink color solution.

Other's test is the treatment with lead acetate

Solution which yellow coloured precipitate. And the third one is the dilution with the sodium hydroxide which too gives yellow colorations and which decolorizes on addition of acid.

Test for the presence of Sterols (Steroids)

Liebermann reaction 2ml of extract is mixed with CHCl3. 1-2 ml of acetic a hydric and conc. H2SO4(2 drops) were added by slanting the test tube. Gives red, blue and finally red color.

Liebermanns reaction

3 ml of acetic anhydride is mixed with 3ml of extract. Heated and cooled. Few drops of conc. H2SO4 added gives blue color. Presence of Tannin and phenolic constituents tested: Ferric chlorides test, Sample extract gives deep blue-black color.

Lead acetate test

Sample extract gives white precipitate with lead acetate solution. Gelatin test Sample extract gives white precipitate with gelatin solution.

Thin Layer Chromatography

Silica gel G was used for TLC preparation, and then air dried. Plates were activated by drying in hot air oven at 100 degree C for 1 hr. Extracts from different solvents was spotted over TLC plates. For rapid screening plates were dried and developed in suitable solvents. Detector of TLC plate was done with iodine chamber and UV chamber. From value of different spots available is calculated by using formula.

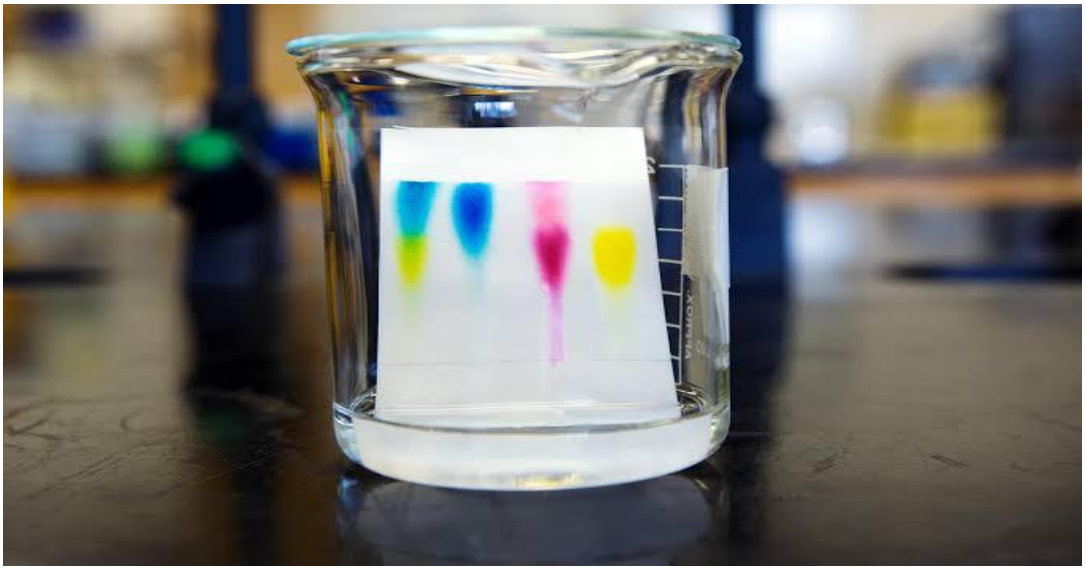

Fig. 4.5 TLC.

Rf value = distance travelled by solute/ distance travelled by the solvent

Fig. 4.6 Visible under the U.V. Chamber.

PHYSIOCHEMICAL STUDIES

Moisture content

About 20g sample was oven dried at 800C until desired weight was obtained after that the sample left for cooling in desiccators for 30min and weight was taken and moisture content was calculated using weight difference.

Content of moisture = initial weight – oven weight/initial weight *100 (* means multiplication)

Acid Value

A dried conical flask was taken to dissolve 5g of oil in 25ml of absolute ethanol, add 3 drops of phenolphthalein indicator. The content of flask was heated over a water bath for 10 min and after cooling the mixture is titrated with 0.1 N KOH.

Calculation of acid value

Acid value = 5.61 n/w

Abbreviations as a explained,

n = ml of 0.1 N KOH required

w = weight of oil in grams

Saponification Value

25ml of 0.5M ethanolic KOH, 2gram of oil were introduced and is refluxed for 30 min to mix well. Add phenolphthalein indicator and titrate straight away with 0.5M Hydrochloric acid. By omitting the oil blank titration is done, then saponification value was calculated. Formula used:

Saponification value = 28.05 (B - A) / W

Abbreviations explained as,

W = weight in g of oil

B = burette value during blank titration

A= burette value during oil titration.

Iodine value

In 250ml iodine flask accurately weighed oil was placed and dissolved in carbon tetrachloride 20ml of wijh reagent added and the flask was covered with stopper and allows to stand in darkening conditions at 25-degree Celsius fir 30 mins. Then 15 min of 15 % potassium Iodine was put on and titrations against 0.1 M Na2S2O3 (sodium thiosulphate) and starch is used as

indicator. After that to calculate iodine value a blank titration was done by omitting the oil A blank titration is also carried out omitting the oil.

Formula used

Value of iodine = 1.269(B-A)/W

Abbreviations as explained,

W = weight in gm of oil

B = burette value during blank titration

A = burette value during oil titration

DPPH (1, 1-Diphenyl, 2-picryl-hydrazyl) Antiradical Activity

Methanolic solution of methanolic extract (20, 40, 60, 80, 100 µg/ ml) was mixed with 400 µM DPPH methanol solution at a ratio 1:3. Gallic acid is taken as standard. A control was prepared using methanol and DPPH solution. The mixture was mixed well and set in dark at room temperature for 30min. The change of colour from violet to yellow of DPPH was determined by measuring the absorbance at 517nm. The percentage of inhibition was calculated by the given formula and also IC50 Value is calculated.

(%) Scavenging activity = Control absorbance - Test absorbance x 100 / Control Absorbance

RESULT AND DISCUSSION

Oragnoleptic Study

Sr. No.	Parameters	Characteristics features
1.	**Color**	Yellowish white
2.	**Odor**	Characteristic
3.	**Test**	Sweet
4.	**Nature**	Coarse powder

Extract Characteristics

Type of Solvent	Consistency	Color	Extractive Value % W/W
Hexane	Oily	Brown	**90.11**
Chloroform	Pasty	Light green	**40**
Methanol	Pasty	Yellowish	**33**
Ethanol	Pasty	Light brown	**92**
Water	Sticky	Light green	**42**

Phyto-Chemical Screening

Plant constituents test/reagent used	Hexane	Chloroform	Methanol	Ethanol	Water
1- Alkaloids a-Meyer's reagent b- dragondroff's reagent	+	+	-	+	+
2- Carbohydrates & glycosides a-Fehling solution b-Molischsolution c-benedict soln.	-	-	+	+	+
3- Phytosterols a-Libermann's test b-Burchard test	+	-	-	-	+
Fixed oil & fat a- Spot test b- Saponification test	+	+	+	-	+
Saponins Foam test Heamolysis test	-	-	-	+	+
Phenolic compound and tannins a- Ferric test b- Gelatin soln. c- Lead acetate sol. d- Acquos bromine solution.	+	+	+	+	+
Proteins&amino acids a- Millian's reagent b- Biurete test c- Ninhydrin test	-	-	+	+	+
Gum and musilage a- Alcoholic test b- Precipitation test c- Molisch test	-	-	-	+	+

Physico Chemical Parameter

Sr. No.	Physico Chemical parameters	Value
1.	Iodine value	212.02 mg/koh/gm
2.	Acid value	9.856 n/w
3.	Saponification value	188 mg/100gm
4.	Moisture	8.575gm

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