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Nutraceutical and Therapeutic Potential of Ethnobotanically Used Bullock's Heart Plant

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Abstract
Annona reticulata L. (Annonaceae) is a small deciduous highly important plant with medicinal value. The fruit is edible, complete in food composition basically protein, vitamin, carbohydrate, seed with oil and with abundant secondary metabolites and principal source of therapeutically active compounds. Fruits showed enough amount of nutrient like total carbohydrate (24.8 gm/100 gm), total phosphorus (18 mg/100 gm) and vitamin C (17.5 mg/100 gm) etc. Mineral analysis of fruit sample showed abundant amount of mineral elements like K, Ca, Mg, Na, and Fe which showed that fruit is good source of minerals and can be used as nutritional purposes. The quantitative determination of phytochemicals showed that the saponins (4.52±0.03%), tannins (3.9±0.5%), terpenoids (2.91±0.4%), phenolics (15.45±0.32 mg/gm), flavonoids (7.45±0.21 mg/gm) and flavanols (1.4±0.4 mg/gm). These values showed that fruits have good source of antioxidant.

Keywords
Ethnobotanical, Nutraceutical, Therapeutic, Flavanols, Antioxidant.

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INTRODUCTION
Annona reticulata L. (**English**: Bullock's Heart, Ox-heart, Netted Custard apple **Hindi**: Ramphal and Mewa) is a small deciduous tree species of Annonaceae, traditionally used for treatment of various ailments. It is native Americas and widely distributed/cultivated in tropical and subtropical region of the world. In India it is cultivated throughout plains and Islands. Near about 137 different species [1] of genus are identified among which most of them are shrub and trees. The extract obtained from various parts of this species possesses medicinal properties and is also used as colouring agent, preservatives, sweetening agents and as an additive in many medicinal formulations [2]. Plants contain abundant amount of secondary metabolites, they are consider to be principal source of therapeutically active compounds along with medicinal formulations. The plant parts have been successfully utilized for development of cosmetics and toiletry preparations [3]. Pinene, myrcene, limonene, terpinen-4-ol, germacrene, were isolated from fruits [4]. The seeds of this plants having series of chemical compounds like michelbine, cytotoxic acetogenins as squamocin, annoreticuin, bullatacin etc [5][6]. The leaves showed antipyretic, antihyperglycemic and antiulcer properties. Bark and root shows analgesic and anticancer property respectively. Seeds having wound healing and antimarking activity [7]. In our continued program aimed at the identification of Ethnonutraceutical drugs leads from natural plant sources, the chemical

composition of the fruits of *A. reticulata* L. was investigated to search for the bioactive constituents by assay of different solvent extraction and isolation by different methods.

MATERIALS AND METHODS:

Collection of plant Materials

The fruit of the plant has been collected from the Baithwalia and adjacent village near Nichlaul block, District Maharajganj, U. P. India. The plant part was washed in double distilled water. The fruit was cut into fine pieces and dried in shade for 10-12 days in order to remove the moisture. The shade dried cut fruit were grinded in mixer grinder and converted into fine powder. It stored airtight container for further experiment.

Chemical Used

All the chemicals used in experiment are of analytical grade.

Preparation of fruit extracts

Soxhlet apparatus was used for extraction. Dried powder of the fruits was subjected for extraction with different solvent (Absolute methanol, absolute ethanol, acetone, diethyl ether and distilled water). After effective extraction, solvents were concentrated using rotatory evaporator under reduced pressure [8]. The crude extract was weighted, and its percentage yield was determined.

Qualitative analysis of phytochemicals

Standard procedure was followed for qualitative analysis of phytochemicals of flower extracts as described by Trease and Evans 1989 [9], Harborne 1973 [10], and Sofowara 1993 [11].

Test for alkaloids

Mayer's reagent (KI + Hg_2Cl_2 solutions): Few drops of Mayer's reagent was added to the extract, appearance of cream-colored precipitate indicates the presence of alkaloids.

Dragondorff's reagent (excess of KI +$BiNO_3$ solutions): Few drops of Dragondorff's reagent was added to the extract, reddish brown colored precipitate appeared.

Hager's reagent (Picric acid): Few drops of Hager's reagent was added to the extract, appearance of yellow colored precipitate indicates the presence of alkaloids.

Test for glycosides

Keller–Killiani test: One ml of glacial acetic acid containing traces of ferric chloride and 1.0 ml of concentrated sulphuric acid were added to the extract. A reddish brown colour formed at the junction of the 2 layers and the upper layer turned bluish green indicating the presence of glycosides.

Borntrager's test: One ml of benzene and 0.5 ml of dilute ammonia solution were added to the extract; appearance of a reddish pink color indicates glycosides.

Test for flavanoids

Alkaline reagent test: Few drops of NaOH solution were added to the extract. Formation of intense yellow color occur which disappear when concentrated HCl is added.

Test for saponins

Foam Test: Two ml of distilled water was added to the extract and shaken vigorously for 15 minutes. If foam produced persists for ten minutes, it indicates the presence of saponins.

Test for tannins and phenols

Ferric chloride test: One ml of ferric chloride solution was added. Bluish-black colour appeared confirmed the presence of tannins.

Test for steroids and terpenoids

Liebermann Burchard test: One ml of anhydrous acetic acid and 1ml chloroform was added to the extract and cooled at 0ºC. Then 1.0 drop of concentrated sulphuric acid was added from the side of the test tube. At the junction a brown ring appears between two layers. Formation of lower deep red color indicates the presences of terpenoids, and the upper layer turns green which show the presence of steroids.

Salkowski test: One ml of chloroform and 1.0 ml of sulphuric acid was added. Reddish brown color lower layer showed the presence of steroids while yellow colour upper layer indicated the presence of terpenoids

Test for proteins

Biuret test: One ml of 40 % NaOH solution and two drops of one percent $CuSO_4$ solution were added. Appearance of proteins indicated by violet color.

Ninhydrin test: Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) were added. Appearance of amino acids indicated by purple colour.

Test for carbohydrate

Fehling's test: Fehling's solution was added to the extract and boiled in water bath. Presence of carbohydrates is indicated by appearance brick red precipitate

Benedict's reagent: Benedict's solution was added and boiled in water bath. The presence of carbohydrates is detected by red precipitate.

Quantitative analysis of phytochemicals

Determination of tannins

Tannins content was determined by the procedure of Van-Burden and Robinson 1981 [12]. Sample (500 mg) was weighed into a plastic bottle (50 ml). 50 ml of distilled water was added and shaken for 1 hr. in a mechanical shaker after that it was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of it was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ prepared in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured within 10 min at 605 nm.

Determination of saponins

Procedure of Obadoni and Ochuko 2001 [13] was followed to determine the saponins content. The samples were ground and 20 gm of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hrs with continuous stirring at about 55ºC. The mixture was filtered, and the residue re-extracted with another 200 ml in 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90ºC. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated, and 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight. The saponin content was calculated as percentage.

$$\text{Percentage of Saponin} = \frac{\text{Weight of Residue}}{\text{Weight of sample taken}} \times 100$$

Determination of total flavonols

Total flavonols content was determined using the method of Kumaran and Karunakaran, 2007 [14]. 2 ml of the plant extract (1 mg/ml) was mixed with 2 ml of $AlCl_3$ prepared in ethanol and 3 ml of 50 gm/l sodium acetate solution. The mixture was incubated at 20 0C for 2.5 hrs after which the absorption was measured at 440 nm. Total flavonols content was calculated in terms of quercetin (mg/gm) using the calibration curve.

Determination of terpenoids

Terpenoids content was determined by Ferguson 1956 [15]. Ten gm. of dried flower powdered was taken and soaked in alcohol for 24 hrs. It was filtered and filtrate extracted with petroleum ether; the ether extract was treated as total terpenoids.

RESULT AND DISCUSSION

Table-1, Fig.1 revealed the percentage yield of crude fruit extracts in various solvents which was higher in ethanol (23.70 %) followed by methanol (21.40 %), Acetone (15.61 %), aqueous (18.65 %), chloroform (10.61 %). The crude fruits extract obtained were subjected to various chemical tests for the detection of phytochemicals. The result revealed the presence of tannins, saponins, terpenoids, steroids, phenols, carbohydrates, flavonoids, glycosides and alkaloids were reported in Table-2 and 3. However, their intensity of appearance differs in different solvents were also recorded. The quantitative determination of phytochemicals showed that tannins content was (3.9 ± 0.5 %) which was determined using standard curve equation derived from standard curve of tannic acid. Tannins are potential antioxidants. Which considered to be cardioprotective, anti-inflammatory, anti-carcinogenic and anti-mutagenic. The tannic acid present in extract showed better availability in comparison with other plant extracts. The other biofunctional biomolecules viz. terpenoids (2.91 ± 0.4%), flavanols (1.4 ± 0.4 mg/gm QE) and flavonoid (7.45 ± 0.21 mg/gm CE) in terms of quercitin. However, contents of saponins (4.52 ± 0.3%), total phenolics (15.45 ± 0.32 mg/gm GAE) recorded in Table-5. The availability of terpenoids, flavonols, flavonoids, tannins, saponins and phenolics showed the bio functionality of *A. reticulata* L., which virtually used by local peoples and tribes Nichlaul block of Maharajganj district U.P., India. Plant of *A. reticulata*, fruits ripe and unripe and fruit powder is presented in Fig.2, 3, 4 and Fig. 5.

Table 1: Percentage Yield of Different Extract of *Annona reticulata* L. Fruit.

Solvents Extracts	Colour	Consistency	Percentage Yield (w/w)
Aqueous	Yellow	Non sticky	15.61 %
Methanol	Greenish Yellow	Non sticky	21.40 %
Ethanol	Greenish Yellow	Non sticky	23.70 %
Acetone	Greenish Yellow	Semisolid with sticky	18.65 %
Chloroform	Greenish white	Semisolid	10.61 %

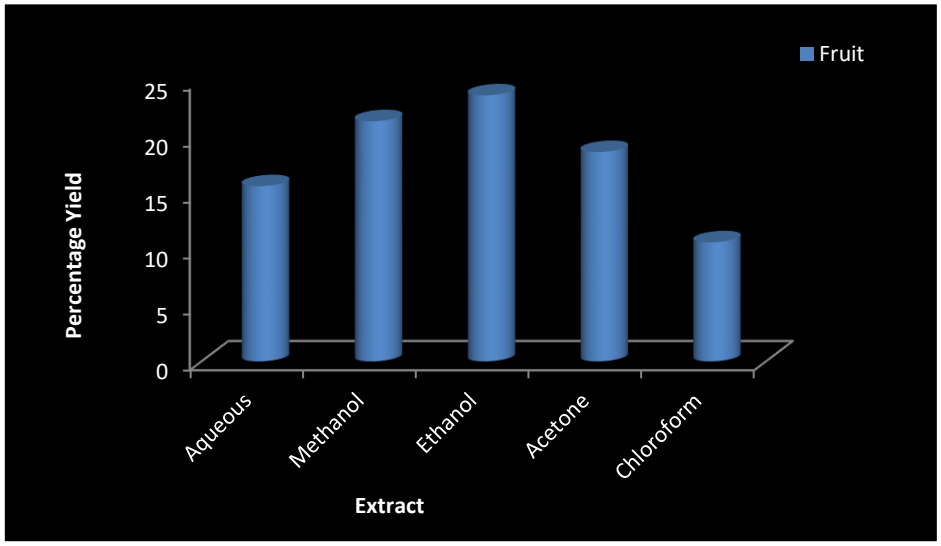

Fig 1: Percentage Yield different Extract of Fruit

Table 2: Nutritional Component of *Annona reticulata* L. fruit.

Nutrient	Dry weight basis (100 gm)
Moisture content (Fresh Sample)	28.3 %
Ash	4.65 %
Acid Insoluble ash	0.854 %
Water soluble ash	0.920 %
Fat	0.9 gm
Total Carbohydrate	24.8 gm
Crude fibre	2.2 gm
Calorific Value	422 KJ
Total phosphorus Content	18 mg
Vitamin C	17.5 mg

Table 3: Preliminary Phytochemical Screening of Fruits of *Annona reticulata* L. fruit

Chemical Constituents	Test	Extracts				
		Aqueous	Methanol	Ethanol	Acetone	Chloroform
Saponins	Foam	++	+++	+++	+	+
Flavanoids	Alkaline	+	+	+	+	+
Steroids	Libermann Burchard	++	++	++	++	++
Terpenoids	Salkowski	+	++	++	++	+
Alkaloids	Mayr's, Hager's	+	+	+	+	+
Glycosides	Keller-Killani, Borntrager	+	+	+++	+	-
Phenolic/Tannins	Ferric-Chloride	+++	+++	++	++	-
Carbohydrate	Benedict's Fehling	+	+	+++	+	+
Proteins	Ninhydrin, Biuret	++	++	+++	++	+
Amino acids	Ninhydrin	++	+	++	++	+

+ = Good, ++ = Better, +++ = Best

Table 4: Mineral Content of *Annona reticulata* L. Fruit Pulp

Mineral Elements	Quantity (mg/100gm) dry basis
Calcium (Ca)	29.5 mg
Iron (Fe)	0.66 mg
Magnesium (Mg)	16.4 mg
Sodium (Na)	3.4 mg
Potassium (K)	378 mg
Copper (Cu)	BDL
Zinc (Zn)	BDL

Table 5: Quantitative Determination of Phytochemical from *Annona reticulata* L. Fruit

Phytochemicals	Fruits Yield
Saponins (%)	4.52 ± 0.3
Tannins (%)	3.9 ± 0.5
Terpenoids (%)	2.91 ± 0.4
Phenolics (mg/gm GAE)	15.45 ± 0.32
Flavanoid (mg/gm QE)	7.45 ± 0.21
Flavanols (mg/gm CE)	1.4 ± 0.4

Table 6: Secondary Metabolites and Their Role in the Human Body

S. No.	Biofunctional/Bioactive molecule	Activities in Biological System
1.	Sesquiterpene, Terpenoids fraction	Work as central and peripheral analgesic and anti-inflammatory activities.
2.	Ethanolic extract	Wound healing potential and antimarking potential in combination with neem oil.
3.	Methanolic extract	Work as glucose tolerance test
4.	Phenolics, polyphenol (tannins)	Antioxidant properties, anti-inflammatory response in body, astringency and prevent predation.
5.	Flavonoids	It displays antioxidant, anti-inflammatory and anti-allergic properties.

Fig 2: Plant of *Annona reticulata*

Fig 3: Fruit of *Annona reticulata*

Fig 4: Ripe fruit of *Annona reticulata*

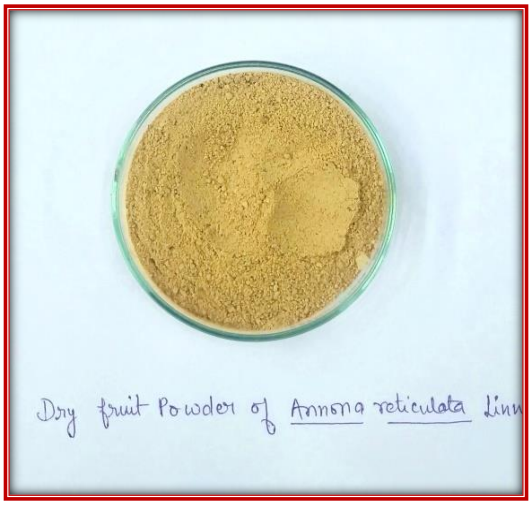

Fig 5: Fruit powder of *Annona*

CONCLUSION

Plants have a noteworthy position in the medicinal field due to their therapeutic properties and prove to be a rich source of drugs. Therapeutic properties are due to the presences of various phytochemicals present in the plant. Plant derived chemical compounds play a key role in the reduction of chronic diseases as well as prominent impact in health benefits. They act as antioxidant, antitumor, anti-inflammatory, anticancerous, anti-diabetics etc. Therefore, exploration is further required for isolation and characterization of phytochemicals.

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