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Phytochemical analysis of custard apple (*Annona squamosa*) and Karanja (*Pangamia pinnata*) seed extracts

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Abstract
Use of ethno-veterinary to cure the ailments is a traditional approach since long and considered as an alternative to chemical anthelmintics where resistance is inevitable. Plant based products are known to have anthelmintic properties and have negligible residue and are eco-friendly. The present study was conducted to ascertain the different phyto-constituents present in the seeds of Custard Apple (*Annona squamosa*) and Karanja (*Pangamia pinnata*). Seeds of the plants were procured and processed under laboratory of the department. Cold aqueous and methanolic extracts were prepared and analyzed further for the presence of different phyto-chemicals, such as alkaloids, tannins, saponins, sterols, fixed oils, proteins, anthraquinones, flavonoids, reducing sugars, glycosides etc. The percentage yield for aqueous and methanolic extracts revealed was higher in *P. pinnata* seed extract as compared to *A. squamosa* seed extract. In acclaimed to consistency of extracts were semi-solid in nature. Blackish to dark brown color was observed in aqueous extracts of *A. squamosa* and *P. pinnata*, whereas yellowish brown color was observed in methanolic extracts of the plants. Identification and quantification of phytochemicals with the help of chromatographic techniques need to be carried out for estimating their anthelmintic properties.

Keywords: *Annona squamosa*, ethno-veterinary, *Pangamia pinnata*, phyto-constituents, seeds

1. Introduction
Since time immemorial, livestock keepers in India have been using traditional medication based on plant formulations which effectively reduce the degree of parasitism (Sri Balaji and Chakravarthi, 2010) [1]. They are cheaper, easily available, biodegradable and having negligible drug residue in the processed product (*i.e.* meat, milk). Till now there is no single report of the development of resistance against plants products (Tariq *et al*., 2009; Sankar, 2021) [2,3]. Anthelmintic from natural sources may play a key role in the treatment of parasitic infections (Sunilson *et al*., 2010) [4]. All parts of plants, including leaves, bark, fruits, flowers and seeds are used in medicinal preparations. Therefore, the plants contain various phyto-constituents namely, alkaloids, tannins, saponins, sterols, fixed oils, glycosides, proteins, phenols, flavonoids, quinones, terpenoids etc. However, many researchers demonstrated the widespread activities of the plants against gastrointestinal parasites of small ruminants (Bauri *et al*., 2015; Veerakumari, 2015; Mazhangara *et al*., 2020) [5,6,7]. *Annona squamosa* belonging to family annonaceae, also known as Custard Apple, Sugar Apple, Sweet sop, Gandagaatra, Sitaphal is a native to South America and the West Indies. It is a small flowering tree of 3 to 5 meter in height and cultivated throughout India. The seeds of the plant are known to possess anthelmintic activity against gastrointestinal strongyles of small ruminants, mainly sheep's and goats (Sachan *et al*., 2015) [8]. The plants is also useful in the destroying lice in the hair and having abortifacient, antimutagenic, scavenging, antimicrobial, antidiabetic, licicidal, hepatoprotective, antithyroid, antigenotoxic antiplasmodial and molluscicidal activity (Bhattacharya *et al*., 2012) [9]. Another important plant, the *Pongamia pinnata* is frequently notable as karanja, kanji, Indian beech is an adaptable tree and has been recognized in different system of traditional medicines. The plant is used for anti-inflammatory, anti-spasmodic, anti-hyperglycaemics, anti-lipidoxidative, anti-diarrhoeal, anti-ulcer, antioxidant etc. In Indian sanskriti their seeds were used for skin aliments and anthelmintic properties (Chopade *et al*., 2008; Sunilson *et al*., 2010) [10, 4]. Therefore, phyto-chemical analysis of the seeds extract of *A. squamosa* and *P. pinnata* was done to provide an exact biochemical basis for their different

phyto-constituents regarding anthelmintic activity.

2. Material and Methods

2.1 Location and place of work

The proposed studies were carried out in the Department of Veterinary Parasitology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur (Madhya Pradesh). The Jabalpur city is located in between latitude 23º 10' N and longitude 79º 56' E with an average height of 411 m above mean sea level.

2.2 Collection and processing of plant material

The seeds of *A. squamosa* and *P. pinnata* were purchased from the local market of Jabalpur (M.P.). The plants materials were identified and authenticated by the botanist in the Department of Botany, Jawahar Lal Nehru Krishi Vishwa Vidhyalaya, Jabalpur. The collected plants materials were cleaned manually to remove adherent impurities and then air-dried under shade at a well-ventilated place in the laboratory (Fig 1). Further drying was done in the incubator at 30ºC to remove remaining moisture. The fully dried seeds of *A. squamosa* and *P. pinnata* were grinded until very minute powdery forms were prepared and finally stored in air tight container. Cold aqueous and methanolic extracts were prepared from the same plant.

Fig 1: Natural shade drying of freshly collected plant materials A) Custard apple seeds B) Karanja seeds

2.2.1 Preparation of aqueous extracts

Fifty gram of powdered sample of each plants material was soaked in 400 ml of distilled water in a glass flask and stirred at hourly intervals initially for 2-3 times and left undisturbed for 8 hour for soaking at room temperature and then filtered through Whatman filter paper No.1 with the help of separating funnel. The obtained filtrate was concentrated by evaporation at 30-35ºC under biological incubator with some modification in procedure as described by Kanojiya *et al.* (2015) [11] and Saiyam, (2018) [12].

2.2.2 Preparation of methanolic extracts

Fifty gram powder from each plant was taken and soaked in 400 ml of analytical grade of methanol in glass flask and stirred properly at every one hour interval covered with aluminum foil at room temperature. The soaking was done for a period of 72 hours. The soaked material were filtered through Whatman filter paper No.1 with separating funnels. The filtrates were concentrated by evaporation at low temperature (30-35 ºC) under biological incubator as described by Kanojiya *et al.* (2015) [11] and Bendigeri, (2019) [13].

2.2.3 Calculation of extractability

The percentage yield of the extracts was calculated as per Kanojiya *et al.* (2015) [11]

$$\text{Extraction yield (\%)} = \frac{(w_1 \text{ X } 100)}{w_2}$$

Where, w_1 = Weight of extract obtained after evaporation the solvent
w_2 = Weight of the plants powder used

2.2.4 Preservation of extracts

The extracts were kept in air tight containers and marked individually and preserved at 4 ºC in refrigerator till further use (Fig 2)

A. B.

Fig 2: Extracts of plants materials A) Custard apple seeds methanolic B) Karanja seeds methanolic

2.3 Phyto-chemical screening

The obtained plants extract residue were tested for the presence of various phyto-chemicals like, alkaloids, tannins, saponins, sterols, fixed oils, proteins, anthraquinones, flavonoids, reducing sugars, glycosides etc (Bendigeri *et al.*, 2019) [14] (Table 1).

Table 1: Phyto-chemical testing of different plants extracts

S. No.	Test	Phyto-chemical Constituents	Observations
1.	Dragendorff's test	Alkaloids	Development of turbidity or precipitation
2.	Wagner's test		Brownish flocculent precipitate
3.	Benedict's test	Glycoside's	Brownish red precipitate
4.	Lead acetate test	Tannin's	Formation of precipitate
5.	Ferric chloride test		Green colouration in the filtrate
6.	Froth test	Saponins	Formation of froth
7.	Sulkowski test	Sterols	Development of red colour in chloroform layer and greenish yellow fluorescence in other layer
8.	Filter strip test	Fixed oil	Appearance of oil base spot
9.	Biuret test	Protein	Violet pink colour formation

10.	Bontrager's test	Anthroquinones	Pink colour in the ammonical layer
11.	Flavonoids test	Flavonoids	Intense yellow changing to colourless on addition of few drops of diluted HCl

3. Results and Discussion

Plants have been studied as potential sources of phyto-chemicals which were used for controlling parasites of both animal and humans due to their anthelmintic properties (Masopha and Masika, 2010) [15]. The detrimental effects caused by gastrointestinal parasites on the development of sheep and goats and decreased response of these gastrointestinal parasites against the chemical anthelmintics have led to numerous studies examining the use of plant extracts as nematode controlling agents (Soares *et al*., 2015) [16].

The aqueous extractability percentage was higher in case of seeds extracts of *P. pinnata* (04.22%) seed as compared to *A. squamosa* (03.97%) seeds. The highest yield in methanolic extraction was observed in *P. pinnata* and *A. squamosa* seeds were 11.72% and 08.73%, respectively. The methanolic extractability of *A. squamosa* seed is lower as compared with the finding of Sachan *et al*. (2015) [8] (42.85%) which could be due to use of the different extraction method. Different solvent extractions and extract fractionations process yield different type and quantity of bioactive compounds from the plant, Sulla (*Hedysarum coronarium*) (Molan *et al*., 2000) [17]. The total action of the extracts is a sum of the activities of their constituents. The aqueous extract of custard apple seed was blackish in color whereas dark brown extract obtained from karanja seed. However, yellowish brown color methanolic extracts with semi solid consistency were obtained from both *A. squamosa* and *P. pinnata* seeds.

A. squamosa seed aqueous extract was positive for most of the active principles except anthraquinones, glycosides, reducing sugars and tannins by ferric chloride test. However, proteins were found in negligible amount in custard apple seed methanolic extract. Therefore, both extract of custard apple seed (aqueous and methanolic) were tested positive for alkaloids, tannins, saponins, sterols, fixed oils and flavonoids (Fig 3-4).

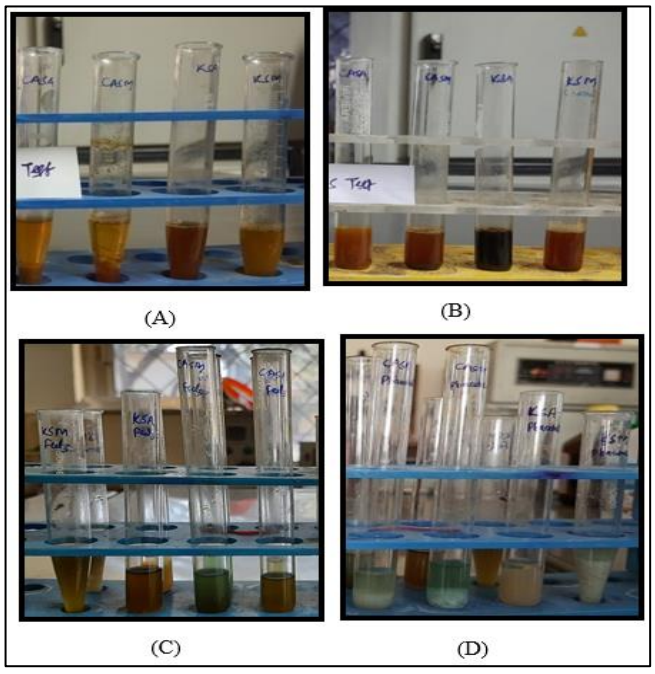

Fig 3: Phyto-chemistry of plants extracts: Tests for alkaloids: A) Dragondorff's test B) Wagner's test Tests for tannins: C) Ferric chloride test D) Lead acetate test

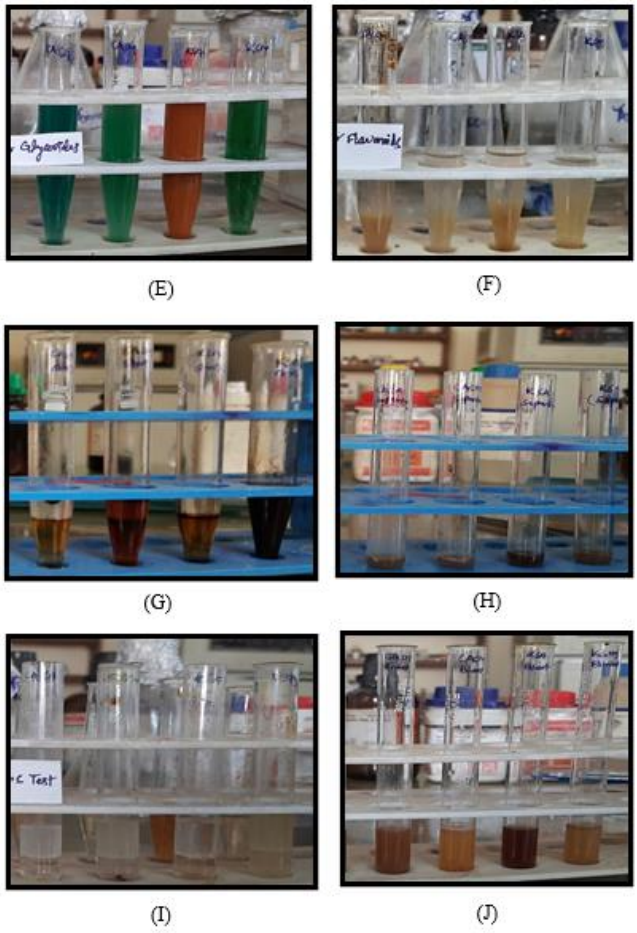

Fig 4: Phyto-chemistry of plants extracts E) Glycosides (Benedict's test) F) Flavonoids G) Sterols (Sulkowski test) H) Saponins (Foam test) I) Anthraquinones (Bontrager's test) J) Proteins (Biuret test)

Our findings are almost in agreement with findings of Sachan *et al*. (2015) [8] however, proteins were detected in aqueous extract in our study. In another study, Kamraj and Rahuman (2011) [18] revealed the presence of condensed tannins and alkaloids in leaf methanol extracts of *A. squamosa* are accordance to our findings of custard apple seed methanolic extracts. Pandey and Barve (2011) [19] isolated 30 acetogenins from *A. squamosa* Linn seeds *e.g.* squamocins, coumarinoligans, annotemoyin-1, 2, squamocin, cholesteryl, glucopyranoside and flavonoids. The presence of flavonoids in the plant extracts affect the moulting as well as the survival of various larvae and potentiates the activity of various other drugs, chemicals etc (Srivastava *et al*., 2011) [20].

Phyto-chemistry of *P. pinnata* aqueous extract was tested positive for alkaloids (Dragendroff's test), tannins (lead acetate test), sterols (Sulkowski test), fixed oils (filter strip test), proteins (biuret test), glycosides and reducing sugars (Benedict's test) and additionally was found positive for flavonoids in both type of extracts. Methanolic extract tested positive for both the tests for alkaloids (Dragendroff's test, Wagner's test) and tannins (lead acetate test, ferric chloride test). Saponins, fixed oils, anthraquinones were exemplified positive with foam test, filter strip test and bontrager's test, respectively (Fig 3-4). Sarma and Venkatesh (2017) [21] studied the phyto-chemistry of aqueous and methanolic leaves extracts of *P. pinnata* and revealed similar finding of alkaloids, tannins, flavonoids, glycosides and sterols. In

present findings, sterols, proteins, glycosides and reducing sugars were detected in aqueous extracts whereas anthraquinones was detected in methanolic extract. Maestrini *et al*. (2020) [22] studied that *Medicago* spp. contains saponins and prosapogenins have *in vitro* inhibiting effects against sheep gastrointestinal strongyle eggs, although with a different level of efficacy. Saponins affect the cell wall integrity interacts with the collagen of the cuticle of the nematodes where by the cell will lose electrolytes and chemicals leading to the flushing of the parasite from gastrointestinal tracts (Doligalska *et al*., 2011) [23]. Furthermore, Fugare *et al*. (2021) [24] demonstrated various phyto-constituents from seeds oils of *P. pinnata*, as Karanjin, Pongamol, Glabrachalcone, Kanjone, Pongapin etc are distinguishable finding from our study reported. Besides, alkaloids found in our study which are nitrogenous substances exhibiting excellent anthelmintic activity, but their biochemical mode of action is not clearly understood and has been likened to that of tannins (Fumum *et al*., 2017) [25]. However, Cabardo and Portugaliza (2017) [26] detected tannins in the ethanolic extract and saponins in the aqueous extract as the primary metabolites in *Moringa oleifera* seeds. Anthelmintic activity of Quebracho extracts may be due to condensed tannin which decreases the viability of the larval stages of the gastrointestinal nematode parasites and also interferes with egg hatching and development to infective larval stage (Athanasiadou *et al*., 2001) [27].

Table 2: Phyto-chemical constituents of different plants extracts

Phyto-chemical parameter		Aqueous and methanolic plants extracts			
Active principle	**Phytochemical test**	**CASA**	**CASM**	**KSA**	**KSM**
Alkaloids	Dragendroff's test	+	+	+	+
	Wagner's test	+	+	-	+
Tannins	Lead acetate test	+	+	+	+
	Ferric chloride test	-	+	-	+
Saponins	Foam test	+	+	-	+
Sterols	Sulkowski test	+	+	+	-
Fixed oils	Filter strip test	+	+	+	+
Proteins	Biuret test	+	-	+	-
Anthraquinones	Bontragers's test	-	-	-	+
Flavonoids	-	+	+	+	+
Glycosides	Benedict's test	-	-	+	-
Reducing sugars	Benedict's test	-	-	+	-

(Where, CASA: Custard apple seed aqueous, CASM: custard apple seed methanolic, KSA: Karanja seed aqueous, KSM: Karanja seed methanolic, '+' indicate positive test, '-' indicate negative test)

Alkaloids were detected in all extracts of the selected plants except karanja seed aqueous (KSA) extract. Tannins were confirmed by lead acetate test in all plants extracts used, whereas ferric chloride test detected tannins in methanolic seeds extracts of custard apple and karanja. The foam test confirmed presence of saponins in all plants extracts used except aqueous seed extracts of karanja. Custard apple seed aqueous (CASA), Custard apple seed methanolic (CASM) and KSA were found positive for sterols with Sulkowski test. The filter paper strip test was used for detection of fixed oils in used plants extracts. Therefore, both types of extracts prepared from seeds showed positive test for fixed oil. The presences of proteins were confirmed by Biuret test in extracts of CASA and KSA showed positive test (Fig 3-4). The karanja seed methanolic (KSM) extracts were found positive for anthraquinones with Bontrager's test. The flavonoids compounds were detected in extracts of CASA, CASM, KSA and KSM. Glycosides and reducing sugars were found to be present in KSA extracts.

4. Conclusions

The extractability percentage was higher for *P. pinnata* seed extracts (aqueous and methanolic) as compared to *A. squamosa*. Both the methanolic and aqueous extracts of *A. squamosa* had alkaloids, tannins, saponins, sterols, fixed oils and flavonoids as common constituents whereas the aqueous extract had proteins as an additional component. However, the methanolic and aqueous extracts of *P. pinnata* seed had alkaloids, tannins, fixed oils and flavonoids as ordinary findings whereas the aqueous extract had sterols, proteins, glycosides and reducing sugars as an additional components besides saponins and anthaquinones in the methanolic counterpart. Blackish color was observed in aqueous extract of custard apple seed while dark brown color seen in karanja seed. In methanolic extracts yellowish brown color with semi solid consistency were obtained from both *A. squamosa* and *P. pinnata* seeds. Phyto-chemicals of seed extracts should be further isolated, identified, purified, fractionized and characterized with the help of standardized chromatographic techniques to estimate their anthelmintic properties.

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