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PHYTOPHARMACOLOGICAL EVALUATION OF *ANACARDIUM OCCIDENTALE* L. TESTA FROM CASHEW PROCESSING BIOWASTE

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ABSTRACT

The present study was performed during the year 2024 to evaluate and explore the phytopharmacological properties of *Anacardium occidentale L*. nut testa – a biowaste obtained after cashew kernel processing. The presence of versatile forms of phytochemicals was confirmed by qualitative analysis, whereas anti-nutritional factor analysis revealed no detectable presence.The total phenolic content was quantified, as phenols can contribute to various biological activities. The pharmacological properties of the testawere assessed using in-vitro alpha amylase inhibition assay and in-vitro protein denaturation assay. The former method was used to estimate the anti-diabetic potential and the resultant IC_{50} value of the standard and extract were 1.77±0.145µg/ml and 2.56 ±0.211µg/ml respectively. The latter method was opted to evaluate the anti-inflammatory potential of the extract. The IC_{50} values of the standard was 3.17 ±0.221 µg/mland the extract were 4.80 ±0.314 µg/ml. The results of the study showed that the sample contains substantial amounts of phenols, potentially contributing to the significant anti-diabetic and anti-inflammatory activity compared to that of the standards used.Thus, the study aids the research in utilizing cashew nut testa for developing value added products with pharmacological potential.

Keywords: *Anacardium occidentale L*. testa, Anti-diabetic activity, Anti-inflammatory activity, Biowaste, IC_{50} values, Phytopharmacology.

INTRODUCTION

Cashew cultivation in India has been spread across the peninsular areas including Kerala, Goa, Karnataka, Maharashtra, Tamil Nadu**,**Andhra Pradesh, West Bengal and Orissa. Cashew is also cultivated in other states of the country to a small extent. (Directorate of cashew and cocoa development, Government of India, 2024). As per the report of Kerala State Agency for the Expansion of Cashew Cultivation (2022), about 8,80,000 numbers of high yielding varieties of cashew were developed by Kerala Agricultural University and the same was distributed by Indian Council of Agricultural Research (ICAR) under Cashew Model Garden, Cashew Model Farm and Muttathoru Kasumavu schemes in the year 2022 -2023. Now, contribution of Kerala towards the cashew processing sector in India has set a standard.

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Unit operations in cashew industry follows the steps including roasting, shelling, peeling and packing of kernels. After these processes, the outer shell and testa are discarded as waste from these industries. The bio wastes obtained after processing are utilised to extract cashew nut shell liquid (CNSL) and tannins. The focus area of the present study is to evaluate the phytopharmacological properties of cashew nut testa and todetermine the reusing potential of testa to obtain products that can manage various non- communicable diseases.

MATERIAL AND METHODS

Sample Collection and preparation

The sample in sealed cover were collected from a Cashew processing unit, located at Cheerankavu, Kollam district, Kerala. The sample was grounded and sieved to obtain evenly sized powder. The sieved powder about 200gms was packed and subjected to soxhlet extraction. The extraction was successfully completed at 50^0C for 48 hours using petroleum ether followed by ethanol as solvents. The extracts thus obtained were concentrated at 40^0C using rotary evaporator after successive collection from the soxhlet apparatus. The extracts were then refrigerated at 4^0C for further analysis.

Percentage yield of the sample extract

The estimation of percentage yield of the sample extract from the raw material is relevant in product manufacturing industry. Many factors including the origin of the sample as well as extraction methods can influence the yield of the sample. The percentage yield of the sample was calculated using the following formula.

$$\text{Percentage yield of the sample} = \frac{\text{Weight of the crude extract}}{\text{Weight of the dry sample used for extraction}} = 100$$

Phytochemical screening of the extracts

The petroleum ether extract and ethanolic extract of the sample were used to qualitatively analyse the presence of major phytochemicals. Standardized methods were used for the analysis.

Analysis of anti-nutritional factors in the crude extract

Antinutritional factors are chemical compounds present in plants that are proven to reduce the biological availability of essential nutrients like proteins. These compounds can also be a major reason for disorders caused due to micronutrient deficiency (Samtiya *et al.,* 2020). Thus, the testa sample was analysed to detect the presence of antinutritional factors including phytates,oxalates and nitrates.

Quantitative estimation of total phenolic content

Phenolic compounds are proved to exhibit anti-diabetic activity and inhibit pro-inflammatory cytokine synthesis, contributing to the regulation of immune response. (de Paulo Farias *et al.,* 2021; Yahfoufi *et al.,* 2018). Thus, the total phenolic content of the crude ethanolic extract was determined using Folin – Ciocalteau assay.

Antidiabetic activity of the crude ethanolic extract

Crude ethanolic extract of the sample was evaluated for its antidiabetic activity performing alpha-amylase inhibition assay using 3,5 – dinitrosalicylic acid (DNSA) described by Wickramaratne *et al.,* (2016) with modification. The buffer was solubilized with alpha-amylase from porcine pancreas. Stock solution of sample with standard value was prepared. Starch solution dissolved in

buffersaline, distilled water and sample were mixed well. The test tubes were incubated at 25°C for three minutes after addition of the enzyme.

After the initiation of the reaction the enzyme was further added at one minute interval. In a test tube,dinitrosalicylic acid colour reagent and the mixture was added and placed in a water bath at 85°C–90°C for fifteen minutes. The dilution of the mixture was completed with addition of distilled water. Acarbose which is aná-amylase inhibitor was the standard drug used for the assay.The experiments were done in triplicates. The formula used to calculate the á-amylase inhibitory activity using the spectrophotometric values at 540nm is as follows.

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control - Absorbance of sample]} \times 100}{\text{Absorbance of control}}$$

Anti-inflammatory activity of the crude ethanolic extract

The crude ethanolic extract of the sample wasevaluated for its anti-inflammatory potential using protein denaturation assay described by Bailey-Shaw *et al.,* (2017) with modification. The total volume of reaction mixture was 5ml which consist of albumin solution, sample and phosphate buffer saline. A mixture of standard was also prepared at varying concentrations. The total volume of the control was also set to 5ml. The reaction mixtures were incubated at 37±2°C for thirty minutes and was kept in a water bath at 70±2°C for fifteen minutes. After cooling, the absorbance was measured at 660 nm by a UV/Visible spectrophotometer.The IC_{50} value was determined by plotting percentage inhibition against concentration gradient. The following equation was used to determine the % inhibition of the standard and the sample.

RESULTS AND DISCUSSION

The results of phytochemical screening and assessment of phytopharmacological properties of the crude ethanolic extract of the sample is discussed under the following headings.

Percentage yield of the sample

The weight of the powdered sample taken for soxhlet extraction was 200gms. The table no: 1 illustrated below shows the percentage yield of the crude extracts obtained after soxhlet extraction using petroleum ether and ethanol as solvents.

Qualitative phytochemical analysis

The qualitative analysis of the phytochemicals in petroleum ether extract and ethanolic extract of the sample was performed. The methods and results of qualitative analysis of phytochemicals in the extracts are depicted in table no.2.

The phytochemical screening of the testa using the petroleum ether extract,showed the presence of terpenoids and steroids whereas the ethanolic extract showed the presence of alkaloids, tannins, flavanoids, glycosides, terpenoidsand sugars. Sruthi *et*

Table 1. Percentage yield of the extracts

Sl.No.	Solvent used for the soxhlet extraction	Weight of the powdered sample (g)	Weight of the crude extract (g)	Percentage Yield (%)
1.	Petroleum ether	200	85.43	42.72
2.	Ethanol	200	118.79	59.40

Table 2. Phytochemical present in the crude extracts

Sl.No.	Tests for secondary metabolites	Petroleum ether extract	Ethanolic extract
1.	Alkaloids		
	a) Mayer's Test	b) Dragendroff's Test	a) Negative
	b) Negative	a) Positive	b) Positive
2.	Tannins		
	a) Ferric Chloride Test	b) Lead acetate Test	a) Negative
	b) Negative	a) Positive	b) Positive
3.	Flavonoid's		
	a) Shibata's Test	a) Negative	b) Negative
	b) Ammonia Vapour Test	a) Positive	b) Positive
4.	Saponins		
	Frothing Test	Negative	Negative
5.	Glycosides		
	Fehling's Test	Negative	Positive
6.	Cyanogenic Glycosides		
	Picrate Test	Negative	Positive
7.	Cardiac glycosides		
	Negative	Negative	
8.	Sugars		
	Molisch's Test	Negative	Positive
9.	Anthraquinone's		
	Borntrager's Test	Negative	Negative
10.	Steroids		
	Liebermann's Test	Positive	Negative
11.	Terpenoids		
	a) 2,4 dinitrophenyl hydrazine Test	Positive	Positive
	b) Salkowski test	Positive	Positive

al.(2023) and Zafeer *et al*. (2023) has reported the presence of flavanols and tannins in cashew nut testa which favours the results of the present study.

Analysis of anti-nutritional factors in the crude extract

The sample was analysed to determine the presence of common antinutritional factors. The analysis confirmed the absence of phytates, oxalates and nitrates. The results suggests that the sample may be suitable for formulation of value-added products as there

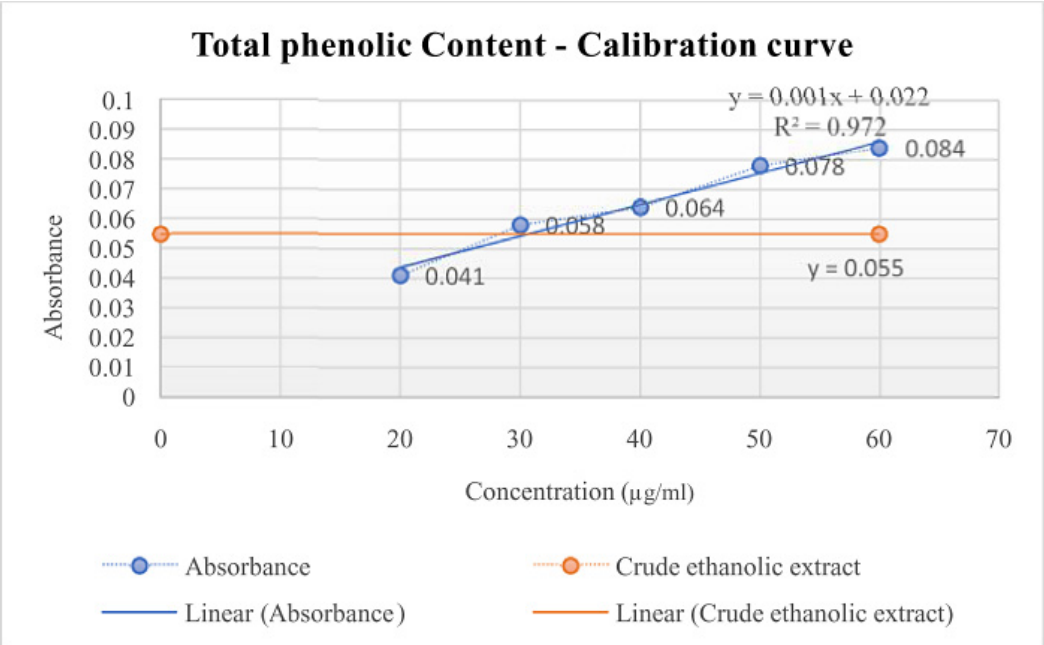

Fig.1. Calibration curve of catechol for determination of total phenolic content

is minimal risk of interference with nutrient metabolism.

Quantitative analysis of total phenolic content of the crude extract

The total phenolic content of the crude extract was determined using Folin- Ciocalteu method. Fig. 1 represents the calibration curve constructed using the standard at different concentration (µg/ml) and corresponding absorbance at 750nm. The estimated value of phenol is represented as mg CE/g of extract.

The concentration vs absorbance graph was plotted to determine the total phenolic content in the ethanolic extract of the sample. A linear regression y = 0.0011x+ 0.0226 with coefficient of determination R^2 = 0.972 was displayed in the above graphical representation. The total phenolic content of the sample was quantified to be29.45+ 0.184µg/mland is expressed as 29.45+ 0.184mg CE/g extract, indicating a substantial amount in the sample and the testa as a comparable source of natural polyphenol.

Table 3. Alpha-amylase inhibitory effects of standard and extract

Concentration (µg/ml)	Average absorbance of standard(nm)	Average % inhibition of standard	Average absorbance of extract (nm)	Average % inhibition of extract
0.2	0.584	41.37	0.674	32.33
0.4	0.471	52.71	0.599	39.86
0.6	0.354	64.46	0.411	58.73
0.8	0.274	72.49	0.324	67.47
1	0.147	85.24	0.221	77.81

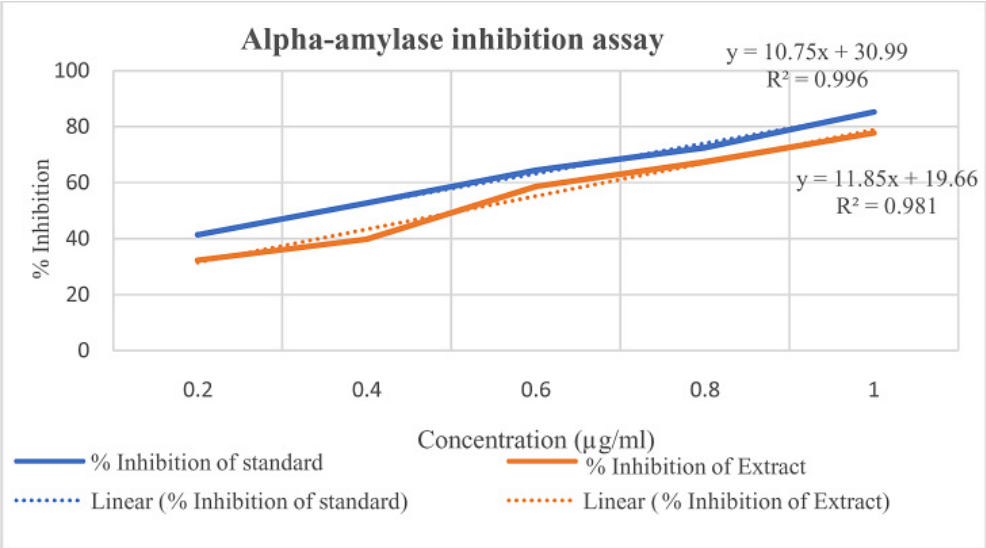

Fig. 2. Alpha - amylase inhibition assay

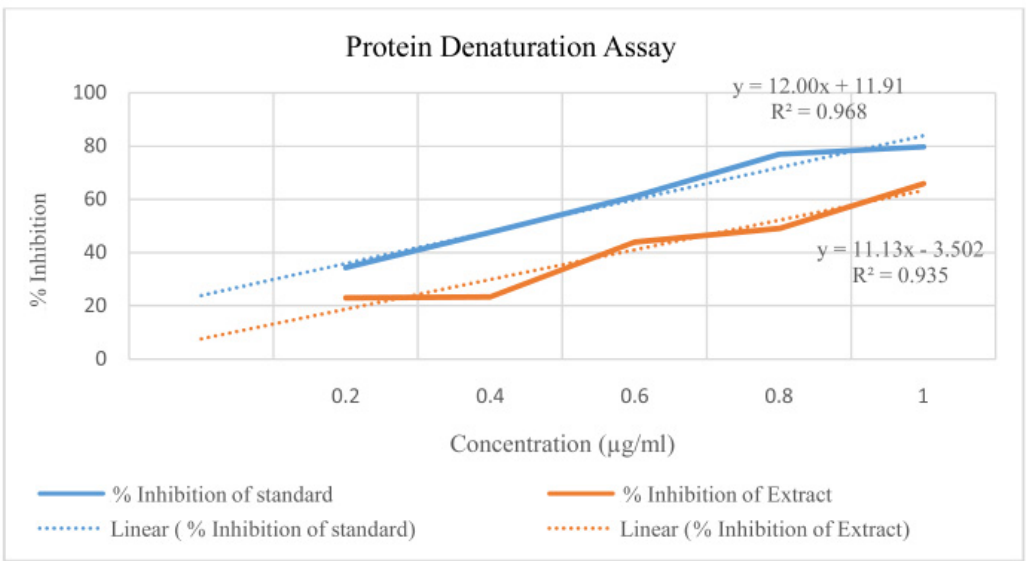

Fig. 3. Protein denaturation assay

Anti- diabetic activity of the crude ethanolic extract of the sample

Anti- diabetic activity of the sample was assessedusing in-vitro alpha amylase inhibition assay. The following tabular column (table no.3) shows the concentration, average absorbance and average percentage inhibition values of the standard and the extract. The graphical representation (fig.2) shows concentration vs average percentage inhibition of the standard and the sample extract. The absorbance of the control was 0.996nm.

It has been reported that the alpha-amylase enzyme is a reason for the rise in post prandial hyperglycemia and blood glucose levels. In order to treat and maintain the blood sugar level, treatments are targeted in inhibiting the activity of this enzyme (Kaur *et al.,* 2021). From the graph, it is evident that there is aconcentration dependent increase in average percentage inhibition proving the antidiabetic potential of the standard and the sample. The regression analysis revealed a strong correlation between both the variables,

Table 4. Effect of standard and extract on protein denaturation

Concentration (µg/ml)	Average absorbance of standard(nm)	Average % inhibition of standard	Average absorbance of extract (nm)	Average % inhibition of extract
0.2	0.599	34.32	0.702	23.02
0.4	0.478	47.59	0.699	23.36
0.6	0.354	61.18	0.511	43.97
0.8	0.211	76.86	0.465	49.01
1	0.185	79.71	0.311	65.90

reflected in the R – squared value of standard (R^2 = 0.9964) and value of sample (R^2 = 0.9814). The concentration dependent inhibition of the standard and sample were determined by calculating the IC_{50} values.The IC_{50} values of the standard and sample were obtained using the prescribed linear regression equation y = mx + c. The resultant IC_{50} of the standard and extract were 1.77±0.145µg/ml and 2.56±0.211µg/ml respectively. From the above results it was evident that the sample possess a very strong alpha-amylase inhibition activity as the IC_{50}value falls below 50µg/ml, supporting the potential of components in the extract as natural anti-diabetic agent.

Anti – inflammatory activity of the crude ethanolic extract of the sample

The protein denaturation assay was used to evaluate the anti – inflammatory activity of the sample. The potential of the components in the ethanolic extract to inhibit the denaturation of bovine serum albumin was determined in comparison to activity expressed by the most potent non – steroidal anti-inflammatory drug, Diclofenac.

The tabular column (table no.4) shows the concentration, average absorbance and average percentage inhibition values of the standard and the extract. The concentration vs average % inhibition graph (fig.3)illustrates the change in absorbance value with respect to the concentration of the standard and the sample. The absorbance of the control was 0.912nm.

From the graph it is evident that there is an increment in average percentage inhibition with respect to the increase in concentration, proving the potential of the standard and the sample to stabilize proteins. The regression analysis revealed a strong correlation between concentration and average percentage inhibition, reflected in the values of R^2 = 0.9688of standard and value of R^2 = 0.935ofsample. The concentration dependent inhibition of the standard and sample were determined by calculating the IC_{50}values.The IC_{50} values of the standard and the extract was3.17 ±0.221 µg/ml and 4.80 ±0.314 µg/ml respectively. Thus, from the results it can be confirmed that the bioactives in the crude ethanolic extract of the sample exhibits significant anti-inflammatory activity in relation to the standard used, supporting its potential use as a natural anti-inflammatory agent.

CONCLUSION

The study systematically evaluated the phytopharmacological profileof cashew nut testa. The qualitative analysis of bioactive compounds was performed both in crude extracts of petroleum ether and ethanol. The results confirmed the presence of key secondary metabolites that possess extensive bioactivity potential. In parallel, analysis of

antinutritional factors resulted in the absence of inhibitory compounds like phytates, oxalates and nitrates,indicating the potential of cashew nut testa for nutritional and therapeutic use. Among the crude extracts,ethanolic extract revealed a broad spectrum of plant derived actives and thus it was used for further analysis.As the presence of phenols is indicative of various physiological effects,the total phenolic content in the crude ethanolic extract was estimated. A significant concentration of phenolics with a value of 29.45+ 0.184mg CE/g extract highlights the pharmacological potential of the sample.The promising phyto-pharmacological properties exhibited by cashew nut testa encourages the efficient recovery, recycling and reuse,to develop cost – effective and sustainable therapeutic as well as value-added products. This approach would also maximize the waste utilisation and ceases unethical use of the testa. In depth clinical studies are suggested to confirm the efficacy of the sample in exhibiting these properties.

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