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Phytochemical Content And Antimicrobial Activity of Cashew Nut Shell Oil

*S.Dorathy Selva Jebapritha[1],S.Karpagam[2]
[1]*Research Scholar, Department of Botany, Queen Mary's College, Chennai.*
[2]*Associate Professor, Department of Botany,Queen Mary's College, Chennai.*
*Corresponding author : *S.Dorathy Selva Jebapritha*

Abstract: *Cashew Nut Shell Oil (CNSO) is a versatile component of the Cashew fruit's nut shell. The CNSO is subjected to preliminary phytochemical screening with ethanol, chloroform, acetone, petroleum ether and aqueous extracts. The antibacterial activity had been tested on the CNSO extract against two Gram positive bacteria namely Staphyloccocus aureus and Enteroccocus faecalis, four Gram negative bacteria namely Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Salmonella typhi. The Antifungal activity had been tested against the growth of organisms such as Epidermophyton floccosum, Aspergillus niger, Penicillium, Microsporum canis Candida albicans and Aspergillus flavus. The extracts were compared with standards like Novobiocin, Amoxicillin and Ketoconazole for antibacterial and antifungal activity respectively. The phytochemical screening showed the presence of many secondary metabolites. All the extracts exhibited the antimicrobial activity against the tested bacterial and fungal species and their zone of inhibition were compared with the standard drugs.*

Keywords: *Cashew Nut Shell Oil, Phytochemical content, Antibacterial and Antifungal activity*

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I. Introduction

Anacardium occidentale L. commonly known as Cashew tree is well known species of Anacardium family. [(1)] In the Tupian languages Acaju means "nut" that produces itself. [(2)] Consumption of nut is associated with lower risk of cardiovascular disease and diabetes.[(3)] Cashew nut shell is about 1/8 inch in thickness and produce oil which is dark reddish brown in colour is resident in a soft honey comb matrix. It is the pericarp fluid of the cashew nut present in between the outer and inner shell (CNSO). The CNSO is widely used for commercial applications in the plastic and resin industries for its phenol content.[(4)] It is cheap and renewable substance that can replace phenol in many applications with equivalent results.[(5)] The oil is used in the manufacture of brake linings and applied to metals as anticorrosive agent.[(6)] The South American natives used CNSO for the treatment of scurry, sores and ringworm. The oil is found to have potent antimicrobial properties.[(7)] CNSO contain approximately anacardic acid 60-65%, cardol 15-20% and cardanol 10% and traces of methyl cardol.[(8)] Gradient elution with tetrahydrofuran and acetonitrite has enabled the polymeric material to be estimated in cashew nut shell liquid. [(9)]

CNSO and its derivatives have been reported to be useful in innumerable applications in polymer based friction, linings, paints, primers, epoxy resins, varnishes and foundry chemicals. [(10)] The biological properties of CNSO includes larvicidal, [(11)] molluscidal, [(12)] antimicrobial. [(13)] antitumour activity, [(14)] antidiabetic, [(15)] and analgesic effects.[(16)] The present study is therefore designed to analyse the phytochemical constituents and antimicrobial activity of various solvent extracts of cashew nut shell liquid.

II. Materials And Methods

The cashew nuts were collected from cashew plantation of Panruti, Cuddalore District, Tamilnadu, India. The seed coat of cashew nut were separated and thoroughly washed with sterile distilled water and dried under shade at room temperature. After the removal of moisture content was ground into fine powder. Grounded cashew nut shells (250g) were heated in the earthern pot for a span of three hours giving a yield of approximately 25 cc of oil. Five separate samples of 10ml of CNSO were extracted with 30ml of ethanol, chloroform, acetone, petroleum ether and aqueous respectively. The mixture is kept for 24 hours and used for further studies.

Phytochemical analysis

Phytochemical screening for qualitative detection of alkaloids are determined by Wagner's test [(17)], carbohydrates by Benedict's test, saponin by foam test, phenols by ferric chloride test, flavanoids by lead acetate

test, aminoacids by ninhydrin test, proteins by Biuret test, diterpenes by copper acetate test, glycosides by modified Borntrager's test, quinones by Con.H_2So_4, terpenoids by Salkowski's test,[18] proteins by biuret test, steroids by Harbourne, cardiac glycosides by Kellerkillani synthesis, oxalate by ethanoic acid glacial,[19] tannins by ferric chloride test, anthocyanin by HCl and NH_3,[20] leucoanthocyanin by isoamyl alcohol , coumarin by Mace method,[21] carboxylic acid by effervescence test and xanthoproteins by HNO_3 [22] test were performed on the five different extracts as described by standard methods of Harborne.[23]

Antibacterial susceptibility test

The antimicrobial activity of five different extracts of the CNSO was investigated by well diffusion method on Mueller Hinton broth.[24] The selected standard strains of bacteria such as *S.aureus, Enterococcus faecalis, E.coli, Klebsiella, Pseudomonas aeruginosa* and *Salmonella typhi* were inoculated into 10ml sterile nutrient broth. Then 6mm diameter wells were made in the inoculated plates. Using a micropipette 30µl, 40µl and 50µl of the solvent extracts were added in the wells. Dimethyl sulphoxide (DMSO) was used as negative control, Novobiocin and Amoxillin were used as positive control. The plates were incubated at 37^0C for 24 hours. Thus, the antibacterial activity was assessed by the diameter of the zone of inhibition and results were recorded.

Antifungal susceptibility test

Fungi namely *Epidermophyton floccosum, A.niger, Penicillium, Microsporum canis, Candida albicans* and *A. flavus* were maintained on Potato Dextrose Agar (PDA). *Invitro* activity were carried out by well diffusion method. PDA was poured into sterile petri plates and allowed to solidify. Wells with diameter of 6mm were made on the plates and different concentrations of extracts 30µl, 40µl and 50µl were loaded in the wells by using micropipette. It was allowed to diffuse for 60 minutes and the plates were incubated at 28^0C for 48 -72 hrs. The zone of inhibition was measured and tabulated.

Results and Discussion

Phytochemical analysis of CNSO showed a variety of rich secondary metabolites such as alkaloids, carbohydrates, saponins, phenols, tannins, flavanoids, aminoacids, diterpenes, terpenoids, proteins, steroids, glycosides, anthocyanin and xanthoproteins. Acetone found to be effective in dissolving the phytochemicals and also it is observed that steroids, glycosides,leucoanthocyanin and coumarin found only in this solvent.(Table - 1)

Table -1 Phytochemical content of cashew nut shell oil

S. no	Name of the solvents / Name of the phytochemicals	Ethanol	Chloroform	Acetone	Petroleum ether	Aqueous
1	Alkaloids	+	+	+	-	-
2	Carbohydrates	+	+	+	+	-
3	Saponins	+	+	+	+	-
4	Phenols	+	+	+	+	+
5	Tannins	+	+	+	+	+
6	Flavanoids	-	-	+	+	+
7	Amino acids	+	+	+	+	+
8	Diterpenes	+	+	+	+	+
9	Glycosides	-	-	-	-	-
10	Quinones	-	-	-	-	-
11	Terpenoids	+	+	+	+	+
12	Proteins	+	-	+	+	+
13	Steroids	-	-	+	-	-
14	Cardiac glycosides	-	-	-	-	-
15	Oxalate	-	-	-	+	-
16	Anthocyanin	-	-	+	+	+
17	Leucoanthocyanin	-	-	+	-	-
18	Coumarin	-	-	+	-	-
19	Carboxylic acid	-	-	-	-	-
20	xanthoproteins	+	-	+	+	+

Note " +" indicates presence and "_" indicates absence of phytoconsituents

Antibacterial activity of CNSO was evaluated against Gram positive and Gram negative bacteria. The inhibition zone of ethanol extract is high against *Staphylococcus aureus, Enterococcus, E.coli* and *Salmonella*. Acetone extract shows high inhibition zone against *Pseudomonas aeruginosa*. The inhibition zone of *Klebsiella pneumoniae* is high in petroleum ether extract. (Table - 2)

Table -2 Antibacterial activity of cashew nut shell oil

Test organisms	Zone of inhibition in mm																			
	Ethanol				Chloroform				Acetone				P.ether				Aqueous			
	C	30 µl	40 µl	50 µl	C	30 µl	40 µl	50 µl	C	30 µl	40 µl	50 µl	C	30 µl	40 µl	50 µl	C	30 µl	40 µl	50 µl
S.aureus	30	21	24	28	30	20	23	26	30	19	21	24	30	9	13	18	30	14	16	19
Enterococcus	26	14	18	21	26	14	17	20	26	14	16	19	26	14	17	20	26	-	9	11
E.coli	24	21	24	26	24	17	19	22	24	14	15	17	24	15	18	21	24	-	-	9
Klebsiella	28	20	22	25	28	20	22	25	28	22	24	25	28	27	30	32	28	9	11	15
Pseudomonas	25	12	17	21	25	10	13	18	25	16	20	23	25	15	18	21	25	13	15	18
Salmonella	30	22	26	29	30	18	21	23	30	20	23	25	30	13	16	19	30	-	9	11

The zone of inhibition is high on the petroleum ether extract of CNSO against all the tested antifungal organisms except *Penicillium*. The inhibition zone of acetone extract is high against *Penicillium*. (Table - 3) The effectiveness of the extracts depends on the concentration that increases the zone of inhibition.

Table -3 Antifungal activity of cashew nut shell oil

Test organisms	Zone of inhibition in mm																			
	Ethanol				Chloroform				Acetone				P.ether				Aqueous			
	C	30 µl	40 µl	50 µl	C	30 µl	40 µl	50 µl	C	30 µl	40 µl	50 µl	C	30 µl	40 µl	50 µl	C	30 µl	40 µl	50 µl
Epidermophyton	20	-	-	9	20	-	-	10	20	11	15	18	20	20	25	28	20	-	9	12
A.niger	19	-	-	10	19	-	-	9	19	-	10	12	19	16	19	22	19	-	-	9
Penecillium	24	9	11	15	24	-	-	9	24	21	25	28	24	18	20	24	24	-	-	9
Microsporum	20	-	-	10	20	9	11	14	20	-	9	12	20	18	23	27	20	-	-	10
C.albicans	20	-	9	12	20	-	-	10	20	-	10	13	20	13	17	21	20	-	-	9
A.flavus	22	-	-	9	22	-	9	11	22	9	12	15	22	17	20	24	22	-	-	9

III. Conclusion

From the present study it is concluded that the CNSO extract has many phytochemical contents and it can be used as a source of antibacterial agent. Pharmacological investigation should be performed by using advanced technique to discover the potential of the Cashew nut sell oil.

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