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Screening of few indigenous plants for the presence of phenols

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Abstract: Plants produce a number of chemical compounds that are used in defence against different pest, and pathogens.(Prakash and Rao1986;1997;Charleston et al.,2004. Higher plants contain a number of secondary metabolites like Phenolics, flavonoid,quinines,tannins,essential oils, alkaloids, saponins and sterols. Tens of thousands of secondary products of plants have been identified and there are estimates that hundreds of thousands of such compounds exist. These secondary metabolites show biological activity, hence plants can be exploited for the discovery of various compounds having pesticidal properties. Among these secondary metabolites Phenols constitute one major group with potency in plant defence mechanism. Plants need phenolic compounds for pigmentation, growth, reproduction, resistance to pathogens and for many other functions. The increased interest in potential biological activity of phenols in plant defence mechanism has led to conduct the present study for the presence of phenolic compounds and their content in various locally available plants i.e Anona squamosa,Clytorea ternatae,Azdirachta indica,Abutilon indicum,Pongamia pinnata and Passiflora foetida with an aim to use these plants as nematicidal plants as phenols are known for resistance against nematodes in plants.

Keywords: Plant secondary metabolites, defence mechanism, phenols, thin layer chromatography, pesticides

I. Introduction

The plant kingdom has the unique character of producing a number of biologically active compounds which are known to play a prominent role in plant defence mechanism against various pests and pathogens. These secondary metabolites include phenolics, flavonoids,quinines,essential oils, alkaloids and sterols. Plants produce a large variety of compounds that contain a phenol group, a hydroxyl functional group on an aromatic ring called phenol, a chemically heterogeneous group also. They could be an important part of the plants defence system against pests and diseases including root parasitic nematodes.(Wuyts et al.,2006) These phenolic –protein interactions are thought to be,in part, responsible for the putative function phenolics as plant defence compounds(Feeny,1976;Rhoades and Cates,1976;Culey,1983;Mole and Waterman,1987)

Extracts prepared from different plants have been reported from time to time to have a variety of properties including insecticidal activity, repellence to pests, antifeedant effects, insect growth regulation, toxicity to nematodes, mites and other agricultural pests and also antifungal, antiviral and antibacterial properties against pathogens.Since Phenols are one of the large group of secondary metabolites which play a major role in plant defence mechanism, the present study was conducted to estimate the total volume of phenols and different types of phenolics are present in the locally available flora with an aim to use these plants as botanical pesticides/nematicides..In the present study six locally plants viz.: Anona squamosa,Clytorea ternatae,Azdirachta indica,Abutilon indicum,Pongamia pinnata and Passiflora foetida were selected.

II. Materials and Methods.

The present study mainly aims to estimate total phenol content in selected plants and also thin layer chromatography analysis of the all the six plant extracts for the presence of various phenolic acids.(qualitative analysis).

A. DETERMINATION OF TOTAL PHENOL:

The total phenol content of the selected plant dry leaf was determined using Folin- Ciocalteu assay method. Total phenolics were determined using the Folin- Ciocalteu reagent Samples (1000mg) were homogenized in 80% aqueous ethanol at room temperature and centrifuged in cold at 10 000 rpm for 15 min and the supernatant was saved. The residue was re-extracted twice with 80% ethanol and supernatants were collected and allowed

for evaporation of ethanol.. Residue was dissolved in 5 mL of distilled water. 200 microlitres of this extract was diluted to 3 mL with water and 0.5 mL of Folin-Ciocalteu reagent was added. After 3 min, 2 mL of 20% of sodium carbonate was added and the contents were mixed thoroughly and the contents were boiled for 1 min in hot water bath. The colour was developed and absorbance was measured at 650 nm in UVD spectrometer after 60 minutes using catechol as a standard. The results were expressed as mg catechol/100 g of dry weight.(Table.1)

The methanol leaf extracts of *Abutilon indicum*, *Azadirachta indica*, *Pongamia pinnata*, *Clitoria ternatae*, and *Paciflora foetida*, *Annona squamosa* were obtained using soxhlett apparatus. To identify the simple phenolic compounds present in the plants extracts thin layer chromatography was performed.. The plant residue was dissolved in ether and then chromatographed two dimensionally on silica gel in ethyl acetate-Hexane. Separation and identification of phenolic acids was performed on precoated silica gel plates. After spotting in plant extracts the plates were developed in Horizontal chambers. After drying the chromatograms were observed in UV Light before and after treatment with ammonia vapor.(Table II)

III. Results and Discussions:

From the Folin- Ciocalteu assay total phenol content in mg of catechol /100g of dry leaf was estimated in all the six plant leaf extracts and the results showed that the total phenol content was ranged between 20 mg to 70 mg in different extracts.(Table I) The highest amount of phenol 70mg catechol/100 g dry weight was observed in *Annona squamosa* followed by *Azadirachta*(64mg), *Clitoria* (52mg), *Pongamia*(34 mg) *Passiflora*(24mg). The lowest amount was observed in *Abutilon indicum*(20mg).

Qualitative analysis of these six plant leaf extracts have shown presence of derivatives of Gallic acid, 3,4 dihydroxy benzoic acid, 2,5, dihydroxy benzoic acid, Syringic acid, Vanillic acid, Salicylic acid and simple phenols like Catechol, Pyrogallol, hydroquinone.(Table II)

A. DISCUSSION:

Plant Phenols play an important role in preventing many diseases and pests. Since phenols have high antioxidant properties isolation of natural phenolic antioxidants from different plant sources help in substituting synthetic antioxidants. .All the six samples chosen have shown presence of Phenols. The results are in conformity with the findings of Savita sagawan et al(2011) who worked on *Pongamia pinnata* and found that The total phenol varied from 7.58 ± 0.15 to 12.2 ± 0.22 mg/gdw in the various extracts like stem, root, seeds and leaf. Gayatri Nahak et al (2010) reported that *Azadirachta indica* and *Melia azedarach* leaves contain phenols and reported that *Melia azedarach* contain more phenol than *Azadirachta indica*. Kandasamy Selvam et al(2012) conducted a study on the presence of total Phenols in the fresh leaves of *Abutilon indicum* growing in hilly as well as plain dry soils and he reported the amount of total phenol as 62 mg/g fresh wt of leaf growing in hilly regions and 45 mg/g fresh wt in dry soil. Manoj Salhan et al (2011) Studied the effect of ethanolic leaf extracts of *Clitoria ternatae* on antihelminthic activity and they reported the presence of phenols in *Clitoria ternatae* leaves. Abdalbasit Adam Mariod et al(2012) reported 75.6 and 93.6 g /Kg extracts of *Annona squamosa* cake and leaf. Sasikala et al.(2011) studied anti oxidant activity of phenolic compounds in *Passiflora foetida* leaves in different solvents and reported that ethanolic peel extract contain higher phenolic content than other extracts.

The present study helps in identification of phenolic compounds in different plants and further Isolation and characterisation of such phenolic compounds may be useful in developing natural antioxidants which are useful in developing botanical pesticides.

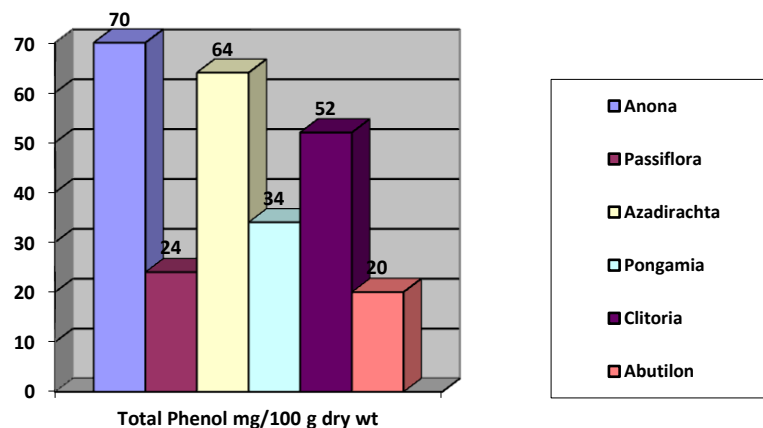
TABLE I: TOTAL PHENOL CONTENT IN DIFFERENT LEAF SAMPLES

S.No.	Name of the plant	Total phenol content (mg catechol eq/100g dry material)
1	<i>Annona squamosa</i>	70mg
2	<i>Azadirachta indica</i>	64mg
3	<i>Pongamia pinnata</i>	34mg
4	<i>Clitoria ternatae</i>	52mg
5	<i>Abutilon indicum</i>	20mg
6	<i>Passiflora foetida</i>	24mg

TABLE: II. QUANTITATIVE ANALYSIS OF LEAF EXTRACTS BY THIN LAYER CHROMATOGRAPHY

Phenolic Acids	Anona			Clitoria			Azadirachta			Abutilon			Pongamia			Passiflora		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Gallic acid	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
3,4-dihydroxy benzoic acid	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
2,5-dihydroxy benzoic acid	-	-	-	-	-	+	-	-	+	-	+	+	-	-	+	-	-	+
Syringic acid	-	-	-	-	-	+	-	-	+	-	+	+	-	-	+	-	-	+
Vanillic acid	-	-	-	-	-	+	-	-	+	-	+	+	-	-	+	-	-	+
Salicylic acid	-	-	-	-	-	+	-	-	+	-	+	+	-	-	+	-	-	+
Simple phenols																		
Catechol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pyrogallol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hydroquinone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
A= FREE PHENOLIC ACID FRACTION, B= AFTER TREATED WITH FOLIN PHENOL, C=FUMING WITH AMMONIA (+ present -absent)																		

Table 1. Total Phenol Content



IV. References

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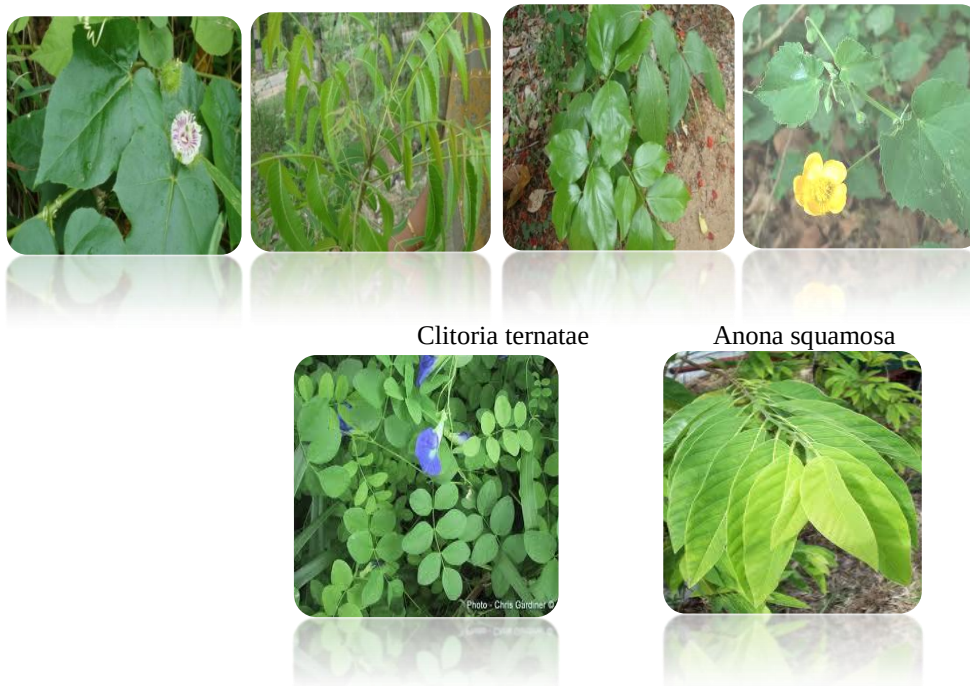
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PLANTS TESTED FOR PHENOLIC CONTENT

Passiflora foetida Azadirachta indica Pongamia pinnata Abutilon indicum



THIN LAYER CHROMATOGRAPHY

