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Phytochemical Analysis and Antifeedant Activity of *Caesalpinia decapetala*

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Chemical composition and antifeedant potential of hexane and methanolic extract as well as essential oil fractions of *Caselpinia decapetala* were investigated. The essential oils from the leaves, flowers and bark of *Caesalpinia decapetala* were obtained by hydrodistillation and analyzed by GC-MS. The highest oil yield was obtained in case of leaves 0.7 mL (0.24 %) which was followed by flowers 0.4 mL (0.11 %) and bark 0.01 mL (0.01 %). Methanolic fraction leads to the isolation of nine compounds. Essential oil samples along with methanolic and hexane extracts were tested for antifeedant activity. All the three samples of essential oils isolated from leaves bark and flowers showed significant activity against *Spodoptera litura*, a polyphagous pest.

Keywords: Essential oils, Antifeedant activity, *Caesalpinia decapetala*, *Spodoptera litura*.

INTRODUCTION

The environmental problems caused by excessive use of pesticides have been the matter of concern in recent years. It has been estimated that about 2.5 million tons of pesticides are used worldwide every year. Plant kingdom is considered as the efficient producer of chemical compounds, which can be used in defense against the pests and diseases. A crude plant extract can exhibit greater overall bioactivity, as it contains a complex mixture of active compounds. This bioactivity can be low in case of individual constituents [1]. The harmful effects of crude plant extracts on insects are manifested in several ways, including toxicity, feeding inhibition [2,3]. In order to find new plant derived chemical which can be utilized as a crop protectant (insecticide, antifeedant and growth inhibitor), screening of plant extract is done [4]. Presently more than 200 plants are known to possess insecticidal properties [5]. Pesticidal properties of various plants have been studied by several authors [6-8]. Extracts from *Azadirachta indica* is antifeedant, antioviposition repellent and growth regulating [9,10]. Pesticides based on plant essential oils could be used in several ways to control large number of pests. Plants essential oils isolated from several medicinal plants found to be effective against *Spodoptera litura*, *Helicoverpa armigara* and *Achaea janata* [11]. Essential oil from *Psoralea corylifolia* Linn. found to be insecticidal and genotoxic against *Culex quinquefasciatus* [12]. Some essential oil constituents, for example, pulegone, limonene, 1,8-cineole and citronellal are active ingredients of commercially available mosquito repellents, flea shampoos and different agrochemicals [13].

Spodoptera litura is an economically important polyphagous pest found throughout the tropical and sub-tropical part of the World. In India, it causes severe damage to a large number of crops including tobacco, caster, groundnut, cotton and other various cruciferous crops [14]. Significant antifeedant activity were shown by extracts of *Syzygium lineare*, *Hyptis suveolens Curculigo orchioides*, *Evolvulus alsinoides*, *Scutellaria scandens*, *Swertia corymbosa* and *Zanthoxylum limonella* against *Spodoptera litura* [15-17]. Leaves of plants *Catharanthus roseus* and *Ocimum sanctum* were also known to exhibit significant activity against fourth instar larvae of *Spodoptera litura* [18].

Caesalpinia decapetala (Roth) commonly known as 'kingri, is a member of the family Caesalpiniaceae. It is widely distributed around the world, found throughout India mainly along ravines of miscellaneous forest to 1800 m [19]. The plant has several medicinal properties namely toxic, astringent, antiseptic, antipyretic, mucilaginous, antidiabetic antitumour and antimicrobial properties [20].

The plant has great phytochemical significance as reveled by literature survey. Variety of secondary metabolites have been reported from *Caesalpinia* [21-23] which can contribute to defense system of plant. In present investigation, we have tested the antifeedant potential of plant extracts, essential oils isolated from leaves, bark and flowers of *Caesalpinia decapetala*

and one of the isolated compound against third instar larvae of *S. litura* (Lapidoptera), a polyphagous pest of groundnut, tomato, cotton, rice, tobacco, castor and legumes.

EXPERIMENTAL

Aerial part of *Caesalpinia decapetala* was collected from Tungnath region of Chamoli district, India at 3200 m height and identified by the taxonomist of Botany Division. Samples were collected at full flowering stage. Voucher specimen was deposited in the Herbarium of Botany Department Hemwati Nandan Bahuguna Garhwal University, Srinagar, India.

All melting points were taken in open capillaries and found to be uncorrected. The ^{1}H and ^{13}C NMR were scanned on Bruker AVANCE 500 MHz at 300, 500 and 125 MHz using deuterated DMSO-d_6, CD_3OD-d_4 and $CDCl_3$ with TMS as internal reference. IR spectra were recorded on Perkin-Elmer Infrared 15 in KBr pellets. Mass spectra were recorded on Micromass Quattro II at 70 eV for EIMS.

GC-MS analysis: The essential oil was subjected to gas chromatographic/mass spectral analysis using a Perkin Elmer make Clarus 500 Gas-Chromatograph equipped with Perkin Elmer Clarus 500 Mass Spectrometry data handling system analytical condition were Rtx®-5 Capillary Column (60 m × 0.32 mm, film thickness 0.25 µm), carrier gas helium (1 mL/min), injector and detector temperature were 210 and 280 ºC, respectively oven temperature was held for 5 min at 50 ºC, then programmed at 3 ºC/min up to 220 ºC and then held isothermal at 220 ºC for 20 min. GC-MS operating in EI mode at 70 eV. The column chromatography was carried out using silica gel (60-120 mesh, Qualigen/Merck). Elute from column chromatography were concentrated under reduced pressure and dried under vacuum. Thin layer chromatography (TLC) was carried out over plates made of silica gel G of Qualigen/Merck.

Extraction and isolation: The fresh leaves (289 g), flowers (360 g) and bark (100 g) were chopped and hydrodistilled in Clevenger-like apparatus for 3.5 h and the obtained essential oil was dried over anhydrous sodium sulphate. Shade dried leaves of *Caesalpinia decapetala* (3.2 kg) were exhaustively extracted with 90 % ethanol. The combined ethanolic extract was concentrated under reduced pressure 50 ºC. The total alcoholic extract concentrate was partitioned between hexane and methanol to give hexane soluble and methanol soluble fraction.

Testing material: Field collected *Spodoptera litura L.* larvae were cultivated in the laboratory at 25 ± 2 ºC and third instar larvae from laboratory culture were used for antifeedant assay.

Assay of antifedant activity (Dual choice leaf disc method): Essential oil and crude extracts were tested against third instar larvae of *Spodoptera litura* L. (Lepidoptera). The dual choice leaf disc method was performed according to the literature [24]. *Ricinius communis* leaves collected from fields were cut in to circular discs (180 cm^2) with the medium vain as marker between two equal halves. Hexane and methanolic extracts and isolated compounds were dissolved in solution, which was sprayed on half of circular leaf disc with 2.5 µg/cm2 concentrations. Other half of the leaf treated with solvent.

Azadirachtin A was used as active control on one half of the leaf [25,26]. Leaf discs were placed in a petri dish (15 cm dia) after drying. Five third instar (freshly moulted) larvae of *S. litura* were placed in the center of leaf and left to feed for 36 h. Five replicates were maintained for every sample. After 36 h the unfed area in the treated and control halves were measured using ΔT area measurement meter. Percent feeding index (PFI) was calculated as:

$$PFI\ (\%) = \frac{\text{Area fed in treated}}{\text{Area fed in treated} + \text{Fed in control}} \times 100$$

RESULTS AND DISCUSSION

The oils were obtained by hydrodistillation of finely chopped fresh leaves (289 g), flowers (360 g) and bark (100 g) of *Caesalpinia decapetala* in Clevenger-like apparatus for 3.5 h to yield colourless pleasant smelling oils and dried over anhydrous sodium. The highest oil yield was obtained in case of leaves 0.7 mL (0.24 %) which was followed by flowers 0.4 mL (0.11 %) and bark 0.01 mL (0.01 %).

Compounds from essential oil were identified on the basis of retention indices (RI), standard samples and by the comparison of their mass spectral fragmentation patterns with against data described in commercial libraries namely NIST and Wiley. A comparative study of the oils composition from leaves, flowers and bark showed similar overall compositions but differences in relative percentages of certain groups. The major components of the leaves were identified as α-pinene (15.13 %), *cis*-ocimene (20.90 %), D-L-limonene (8.14 %), germacrene-D (16.71 %), δ-cadinene (2.12%) while the major components of flowers were α-pinene (16.43%), β-pinene (2.56%), β-myrcene (2.77 %), D-L-limonene (2.68 %), *cis*-ocimene (6.00 %), cryophyllene (4.04 %), germacrene-D (6.67 %) and of bark were D-L-limonene (2.37 %), L-linalool (3.5 %), cryophyllene (2.03 %), 7-(1-methylethenyl)-1-hydroxy-1,4-dimethyl-1,2,4,5-(3H,6H)-octahydroazalene (9.7 %), 1,4-imethylcyclohexane-4-carboxaldehyde (1.66 %) and 4-bromo-1-napthalenamine (2.37 %). The essential oil chemical constituents are listed in Table-1.

The chemical examination of methanol fraction of *Caesalpinia deapetala* led to isolation of nine compounds (Table-2). The compounds **1**, **2**, **3** and **4** were identified as lupeol, betulinic acid, stigmasterol and stigmasterol-3-O-β-D-glucopyranoside, respectively by comparison of co-TLC with authentic samples and NMR data with those of data reported in literature [27-30].

The compound **5** was isolated as colourless crystals from ethanol. Its HR-MS provided the molecular formula $C_{21}H_{28}O_5$ suggesting eight degree of unsaturation. The IR spectrum of the compound exhibited carbonyl absorption at 1728 and 1688 cm^{-1}. Methylation of compound **5** with diazomethane gave a monomethyl derivative 1(a) whose IR spectrum showed no hydroxyl absorption. These facts suggested the presence of a methoxy carbonyl group and a carboxyl group in the compound.

The ^{13}C NMR showed the presence of two carbonyls (δ 180 .9, s and 178.4, s) a methoxyl (δ 51.9, q) and two methyl carbons (δ 17.2, s and 15.1, s). The presence of 2,3- disubstituted furan ring structure in the compound followed from the existence of an oxygen atom and the ^{1}H and ^{13}C NMR signals for a pair of aromatic protons (δ 7.20 & 7.17, J=18 Hz) and four double bonded carbons(δ 148.2, s, 140.7, d, 122.7, s, 109.6, d). The compound was identified as caesaljapin on the basis of data reported in literature [31].

TABLE-1
PERCENTAGE COMPOSITION OF THE ESSENTIAL OILS OF LEAVES, FLOWERS AND BARK OF *Caesalpinia decapetala*

RI	Characterized	m.f.	Leaves (%)	Flowers (%)	Bark (%)
714	α-Pinene	$C_{10}H_{16}$	15.13	16.43	1.18
937	β-Pinene	$C_{10}H_{16}$	1.75	2.56	0.10
1197	*p*-Cymene	$C_{10}H_{14}$	0.17	0.13	0.52
1240	D-L-limonene	$C_{10}H_{16}$	8.14	2.68	2.37
1347	*cis*-Ocimene	$C_{10}H_{16}$	20.90	6.00	0.22
2159	*p*-Menth-1-en-8-ol	$C_{10}H_{18}O$	0.50	0.15	1.37
3206	β-Bourbonene	$C_{15}H_{24}$	0.19	0.21	0.81
3238	β-Elemene	$C_{15}H_{22}$	1.04	3.78	1.62
3574	Aromadendrene	$C_{19}H_{32}$	0.71	0.15	0.66
3661	Germacrene-D	$C_{15}H_{28}$	16.71	6.67	1.98
3757	α-Muurolene	$C_{15}H_{24}$	0.31	0.11	0.814
3811	Curzerene	$C_{16}H_{22}$	0.21	0.55	0.40
3856	δ-Cadinene	$C_{15}H_{24}$	2.12	0.67	0.76
4078	1,5-Epoxysalvial-4-(14)-ene	$C_{17}H_{30}O$	1.78	1.24	0.62
4427	τ-Muurolol	$C_{15}H_{26}O$	0.47	0.61	0.61

TABLE-2
COMPOUNDS ISOLATED FROM METHANOLIC FRACTION OF *Caesalpinia decapetala*

Compounds	m.f.	Characterized
B_1	$C_{30}H_{48}O_3$	Lupeol
B_2	$C_{30}H_{48}O_3$	Betulinic acid
B_3	$C_{29}H_{48}O$	Stigmasterol
B_4	$C_{35}H_{58}O_6$	Stigmasterol-3-*O*-β-D-glucopyranoside
B_5	$C_{21}H_{28}O_5$	Caesaljapin
B_6	$C_{25}H_{38}O_5$	Caesaldecan
B_7	$C_7H_{14}O_6$	Methoxy inocitol
B_8	$C_{16}H_{14}O_4$	4'-Methoxy-4,6'-dihydroxyisoquirtigenin
B_9	$C_{15}H_{10}O_7$	Quercetin

Compounds **6**, **7**, **8** and **9** were identified as caesaldecan, methoxy inocitol, 4'-methoxy-4,6'- dihydroxyisoquirtigenin and quercetin glucopyranoside, respectively by comparison of co-TLC with authentic samples and NMR data with those of data reported in literature [32,33].

For antifeedant activity, crude extracts, one of the isolated compounds methoxy inocetol and essential oils were tested by dual choice leaf disc method to know the percentage feeding index (PFI). Hexane extract showed least Percentage feeding index 62.24 ± 3.12 followed by methoxy inocetol 53.01 ± 5.18 and ethanol extract 51.01 ± 4.28. Maximum antifeedant potential has shown by essential oil isolated from bark 41.49 ± 2.71 (Table-3).

Conclusion

Nine compounds namely lupeol, betulinic acid, stigmasterol, stigmasterol-3-O-β-D-glucopyranoside, caesaljapin, caesaldecan, methoxy inocitol, 4'-methoxy-4,6'- dihydroxyisoquirtigenin and quercetin glucopyranoside were isolated from methanol fraction of leaves of *Caesalpinia decapetala*. The essential oil isolated from leaves, flowers and bark of *Caesalpinia decapetala*, hexane and methanolic fraction of crude plant along with one of the isolated compound were tested for antifeedant activity against *Spodoptera litura*. Essential oils of plant expressed significant antifeedant potential. The results suggest that the studies related to identification and isolation of the active antifeedant constituents present in the plant can be used in eco-friendly formulations for a possible pest control tool.

TABLE-3
ANTIFEEDANT POTENTIAL OF SAMPLES AGAINST *Spodoptera litura* IN TERMS OF PFI

Particular	% Feeding index (PFI) (2.5 µg/cm^2)
Caesalpinia decapetala (hexane fraction)	62.24 ± 3.12
Caesalpinia decapetala (methanolic fraction)	51.01 ± 4.28
Caesalpinia decapetala (essential oil leaf)	42.45 ± 3.74
Caesalpinia decapetala (essential oil flower)	44.53 ± 2.84
Caesalpinia decapetala (essential oil bark)	41.49 ± 2.71
Methoxy inocitol	53.01 ± 5.18
Azadirachtin A	17.89 ± 4.39

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