Phytochemical investigation of *Ruelia patula, Luffa cylindrica* and *Llephantopus scaber*

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Abstract

The phytochemical studies on the plant of *Ruelia patula, Luffa cylindrica and Elephantopus scaber* resulted in isolation of 7-hydroxy 1 methyl coumarin, Stigmasterol, Stigmasterol- 6- en- 3 beta –ol, Campesterol, Dicoumarals, CU I, CUII, CUIII, Lupeol acetate, Triacontanol and Molephantinon are being reported for the first time from this plant. These compounds have been characterized on the basis of spectral and other data.

Keywords : *Elephantopus scaber*, *Luffa cylindrica*,Phyto chemical, *Ruelia patula*

Introduction

Ruellia patula a hairy small undershrubs, found in Arbia, Africa, Srilanka, South west India, Burma and especially in Southern Indian. It belongs to the family *Acanthaceae*. Leaves opposite, subentire or undulate, lineolate. A purplish blue flowers. Seasile or subsessile, solitary or in clusters or racemes. Bracts O; bracteoles large, usually exceeding the calyx. Calyx 5-partite or 5-fid; segments sub equal, narrow, acute, corolla oblique tube ventricose; lobes sub equal, rounded, twisted to the left in bud, spreading in flower. Stamens 4, didynamous; filamnetsglabrous. Ovary 2-celled; ovules 3-10 in each cell; style linear; stigma simple. Fruit a clavate capsule, solid at the base, seed-bearing upwards. Seeds large thin, discoid, margined, hydroscopically hairy, on strong hooked retinacula [1-5]. *Ruellia patula* Jacg was used in the treatment of gonorrhea, syphilis, eye sore, renal infection, cough, wounds, scalds, toothache, stomachache and kidney stones [6-9].

Luffa cylindrica (Linn) M.Roem (Synonym: L.aegyptica ex Hook) Family: *Cucurbitaceae*. The plant is widely distributed through out India. The plant has identified by Dr.Stephen, Dept. of Botany, American College Madurai. The seeds are considered emetic and cathartic. The fruit is used as diuretic and lactogogue [10-14]. The seed oil is reported to be used for skin infections, in the form of tincture the fruit used in the treatment of ascites, jaundice and billiary and intestinal colitis as also in enlarged spleen and liver. The plant is reputed to have anti tubercular and antiseptic properties. The extract of leaves has been used in snake-bites.

The Santals tribesmen use the plant parts in treating convulsions and cramps, tetanus and also in the treatment of syphilis. The fruit is used in dropsy, nephritis, chronic bronchitis [15-17].

Elephantopus scaber Linn, Family *Compositae*, throughout the warmer parts of India, tropical Asia, Australia and America. The whole plant used as Cardiotonic, astringent, febrifuge, diuretic, antidote for snakebite, Root used as antiodotalgic, anti emetic, Leaf used in ulcers, eczema, Root and leaf emollient, anti-diarrhoeal, in dysuria and other urethral complaints, swelling or pain in stomach. Erect, 15-38cmHigh, root stock short, giving off many stout fibrous roots, stem usually dichotomously branched, strigose, with appressed white hairs. Leaves mostly radical, 12.5-20 by 3.8-5,7cm.,forming a spreading rosette on the ground, obovate oblong, rounded or subacute, coarsely serrate-dentate, more or less hairy on both surface, base tapering into an abscure petiole; main nerves numerous, prominent beneath, with reticulate veins between, cauline leaves smaller that the radical, sessile or nearly so. Heads numerous, sessile, closely packed, forming a large at and topped terminal inflorescence nearly 2.5cm across and surrounded at the base by large stiff broadly-ovate cordate conduplicate conspicuously nerved leafy bracts. Involucral bracts in 2 series enclosing 4 flowers, bracts of the outer row half as long as those of the inner, 1-nerved, bracts of inner row usually 3 (rarely 5)nerved, scaruious, linear, cuspidate. Corolla violet, exserted, tube long, slender, limb deeply cleft on one side, causing the 5- liner lobes to present a palmate appearance. Style much exserted, the arms recurved. Pappus white, 1-serrate, consisting of 5(rarely 4) rigid bristles dilated at the base [18-22].

Materials and Methods

The plant parts of *Ruelia patula, Luffa cylindrica and Elephantopus scaber* were collected from Madurai during May 2008. It was authenticated in the department of Botany, The American College, Madurai-2. These plant parts were dried, crushed into a coarse form and extracted. Melting points were recorded on a Veego-Vmp-I apparatus. Infra-red-Spectra were recorded on a shimadzu & FT-IR Perkin Elmer spectrometer by using potassium bromide pellet and nujol mul for a solid and semisolid compounds respectively. NMR spectra were recorded on a H^1 NMR dueteriated chloroform ($CDCl_3$) 500 MHz Tetra methylsilane as internal standard.

Preparation of Column chromatography of Ruelia patula

About 2 gms of ethanol extract was chromatographed over about 20 gms of silica gel (100 – 200 mesh) using hexane, chloroform, ethylacetate in various proportions in order of their increasing polarities. The column was packed by using the suspension of silica gel in hexane. Each 50 ml of the eluate was collected and concentrated. Each fraction was tested for the presence of various constituents and checked on TLC for number and type of constituents. Finally the isolated compounds are separated and labeled as RP_1, RP_2, RP_3, RP_4, RP_5

Preparation of Column chromatography of Luffa cylindrica

After screening the various extracts obtained from 250 grams of coarse seed powder, the petroleum ether extract and benzene extract were found to be promising. The petroleum ether extract (6.2 gms) was dark green gummy residue. The benzene extract was green viscous residue (21 gms) were mixed and chromatographed over silica gel (100-200mesh).
The column was built up by passing two column volumes of n-hexane before the residue was loaded. The solvent was kept 5 cms above the bed and the residue was carefully loaded in the

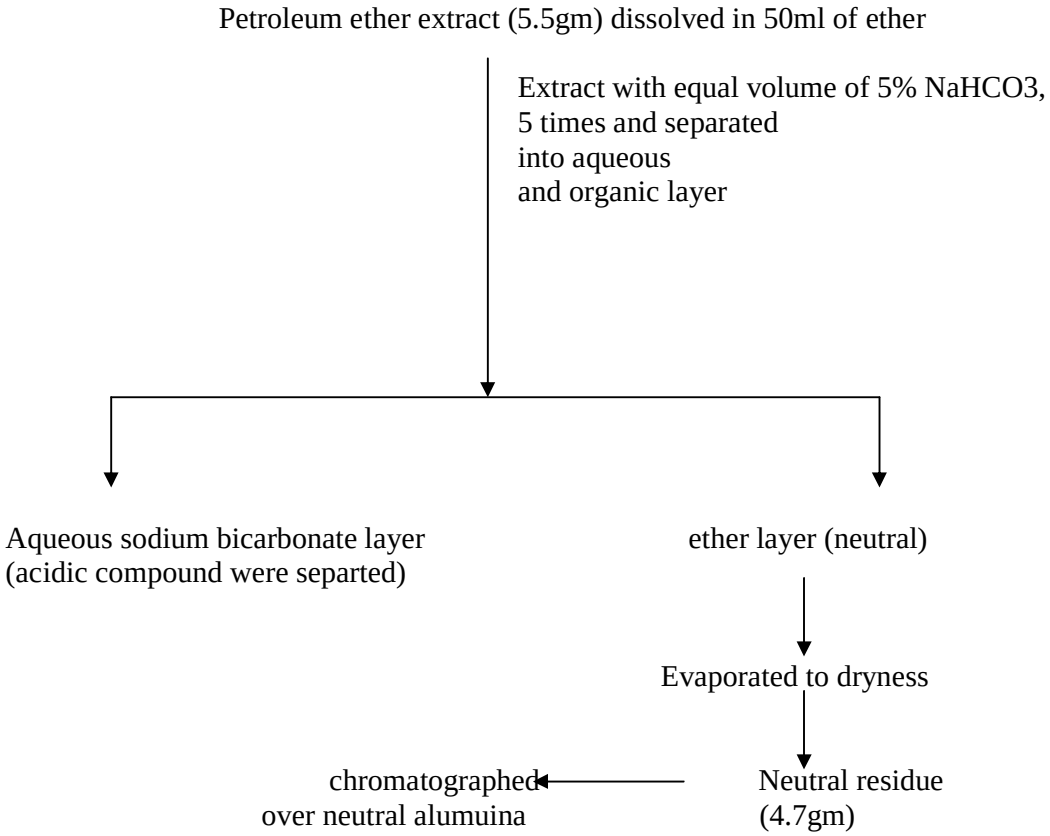
form of a n-hexane slurry. The column was then developed with a series of solvent starting with n-hexane, petroleum ether, benzene, ethyl acetate and methanol.

The different ratio with succeeding solvent were fixed and fractions of 50 ml were collected up to ethyl acetate-methanol system and there after fractions in smaller volumes were collected, checked with T.L.C. and accordingly pooled, concentrated and processed further. The isolated compounds were named as CU-1, CU-2, Cu-3

Preparation of isolated compounds of Elephantopus scaber

About 5.5 gm of petroleum ether extract was separated into acidic and a neutral fraction. The acidic fraction failed to give any crystalline compound chromatography on silica gel.

Separation of acidic and neutral fractions:

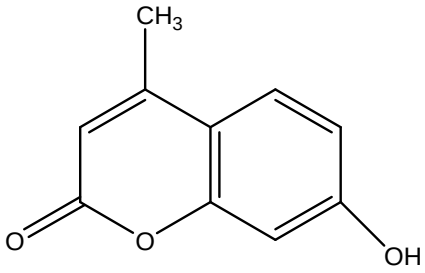


About 4.7 gm of neutral residue of petroleum ether extract was chromatogaphed over about 150gm of neutral alumina using Hexane,Benzene,Chloroform, Ethyl acetae, acetone,methanol and their mixtures in various proportions in order of their increasing polarities. The column was packed by using the suspension of neutral alumina in hexane. Each 100ml of elute were collected and concentrated. The isolated compounds, A, B, C.

Phytochemical investigation [23-25]

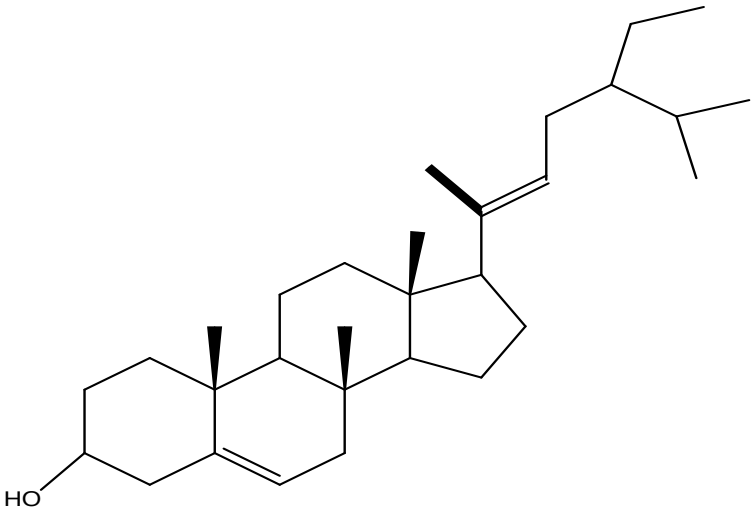
Ruelia patula

Rp-1 (7-Hydroxy-4-Methyl Coumarin)



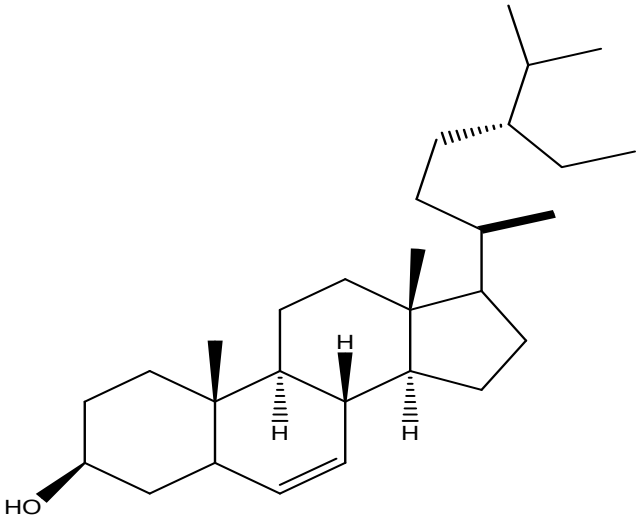
Compound Rp1 crystallized from chloroform-ethylacetate green solid, soluble in chloroform showed a melting point of 182°C gave a Rf value 0.59 was detected by fluorescence spot. The spectroscopic features of these compounds were IIR 3498, 2925, 1670.1276, 1159cm-^{1}, ^{1}HNMR of 1.63, 2.35, 2.42, 6.2δ ppm. It also showed the UV absorption pattern of coumarins λmax 310.

Rp-2 (Stigmosterol)



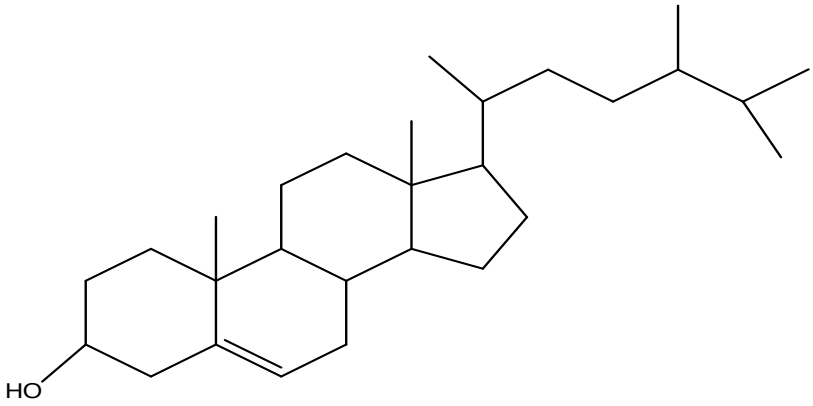
Compound Rp2 crystallized from chloroform yellow semi solid, soluble in chloroform showed a melting point of 140°C gave Rf value 0.76 was detected by Liebermann Burchard test. The spectroscopic features of these compounds were IIR 3534, 2913, 1735.1461cm-^{1}, ^{1}HNMR of 1.1, 2.2, 3.9, 7.2δ ppm [26].

Rp-3 (Stimat-6-en-3-beta-ol)



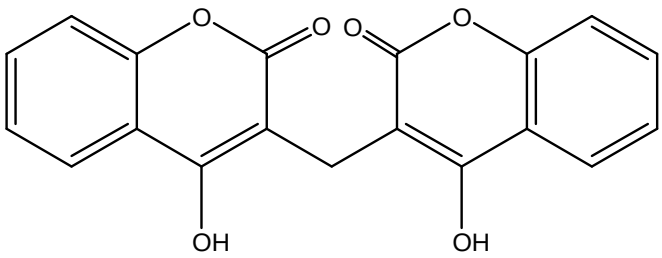
Compound Rp3 crystallized from pet ether-Benzene dark yellow semi solid, soluble in chloroform showed a melting point of 90°C gave a Rf value 0.58 was detected by Liebermann Burchard test. The spectroscopic features of these compounds were IR 3671, 2989, 1720, 1602, 1461cm-^{1}, ^{1}HNMR of 0.9, 1.3, 1.6, 2.0, 7.25δ ppm [27].

Rp4 (Campesterol)



Compound Rp4 crystallized from chloroform-ethyl acetate green semi solid, soluble in chloroform showed a melting point of 90°C gave a Rf value 0.58 was detected by Liebermann Burchard test. The spectroscopic features of these compounds were IR 2927, 1716.1457, 890cm-^{1}, ^{1}HNMR of 0.89, 1.3, 1.8, 2.3δ ppm [28].

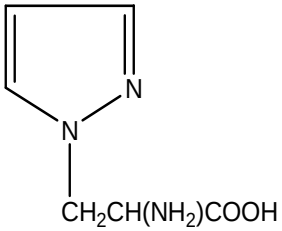
Rp-5 (Dicoumarol)



Compound Rp5 crystallized from methanol brownish green semi solid, soluble in chloroform showed a melting point of 210°C gave a Rf value 0.66 was detected by blue fluorescence spot. The spectroscopic features of these compounds were IR 3694, 2927, 1731.1662, 898cm-^{1}, ^{1}HNMR of 0.88, 1.25, 1.7, 2.0δ ppm. UVabsorption similar to that of coumarin.

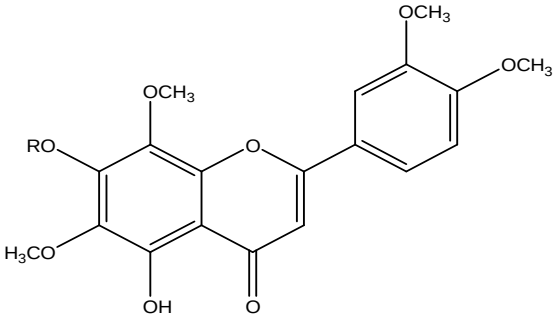
Luffa cylindrica

Cu-I



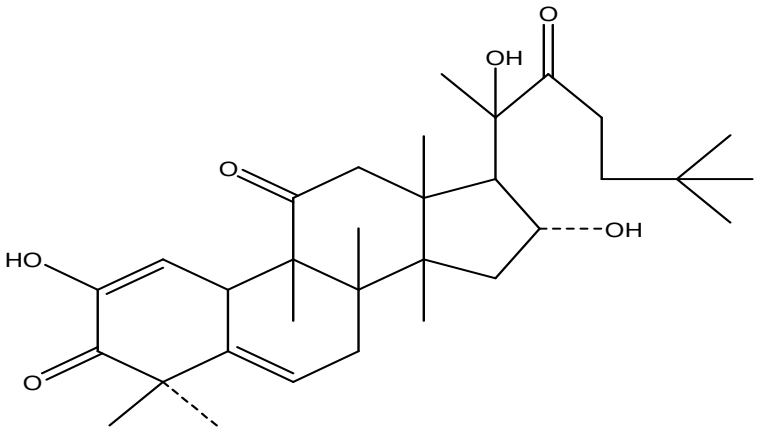
Compound Cu-I crystallized light yellow oil, soluble in hexane, solvent ether, pet ether and benzene gave three dark brown spots (Rf value 0.6695,0.8347 and 0.8921) was detected by using methanolic sulphuric acid translucent test. Emulsification test with water – oil separated, Bile salt solution- dispersed a tiny droplet, stable emulsion. The spectroscopic features of these compounds were IR 2880, 1750.1400-1500, 1190cm-^{1}, ^{1}HNMR of 0.8-0.9, 1.5-1.8, 2.2, 4.1-5.1δ ppm [29].

Cu-II



Compound Cu-II white crystals, melting point 185°C gave Rf value 0.66 was detected by blue fluorescence spot. soluble chloroform gave four spots (Rf value 0.4521,0.5478,0.6695 and 0.9565) was detected by using Libermann Burchard test blue coloured ring [30]. The spectroscopic features of these compounds were IR 3140-3400, 2800,1600, 1380cm-^{1}.

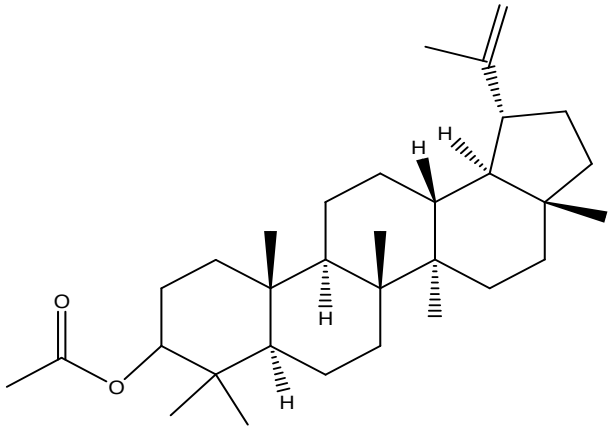
Cu-III



Compound Cu-III green dry powder, melting point 185°C soluble chloroform,gave single spots was detected by using Libermann Burchard test -green blue colour. The spectroscopic features of these compounds were IR 3350-3340, 2900, 1650.1000-1390 cm^{-1}, ^{1}HNMR of 0.65-0.95, 1.2, 2.5, 5.2 ppm [31,32].

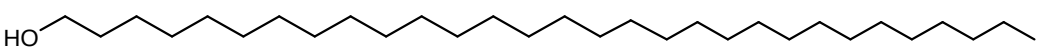
Elephantobus scabar

A. Lupeol Acetate



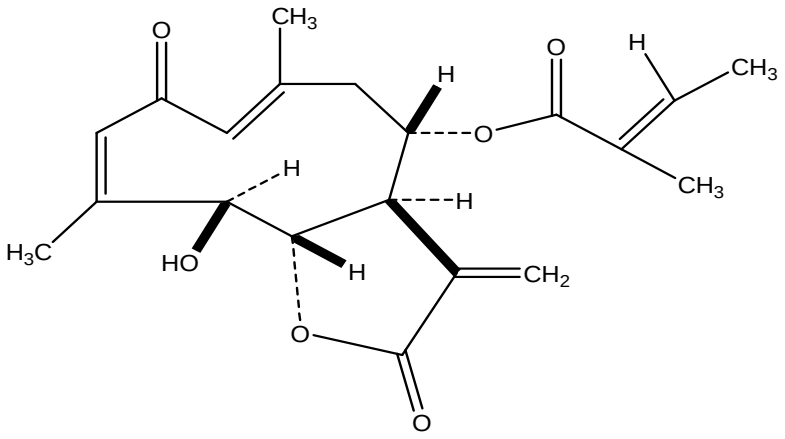
The compound A has a melting point 80-85°C and Rf value 0.912 (hexane:Ethyl acetate (90:10). IR spectra shows band at 2934cm^{-1} aliphatic C-H stretching, 1737cm^{-1} ester stretching, 1464cm^{-1} methylene bending vibration, 1243cm^{-1} OH bending vibration. An NMR spectrum there is a singlet at 1.2δ aliphatic methylene proton.

B Triacontanol



The compound B has a melting point 90-96°C and Rf value 0.901 (Benzene:Ethyl acetate (85:15).IR spectra shows strong band at 2878cm^{-1} aliphatic C-H stretching, 1734cm^{-1} C=O stretching, 1459cm^{-1} methylene bending vibration, the sharp band at730cm^{-1} methylene chain rocking.NMR spectrum there is a singlet at 1.4δ aliphatic methylene proton.

C Molephantinin



The compound C has a melting point 120-127°C and Rf value 0.802 (Chloroform:Acetone (70:30).IR spectra shows strong band at 2914cm-^{1} aliphatic C-H stretching, 1744cm-^{1} ester stretching, 1463cm-^{1} methylene bending vibration.NMR spectrum there is a singlet at 1.5δ aliphatic methylene proton.

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