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Isolation and Characterization of Phytoconstituents obtained from Chloroform Extract *Boswellia serrata* Roxb. Leaves

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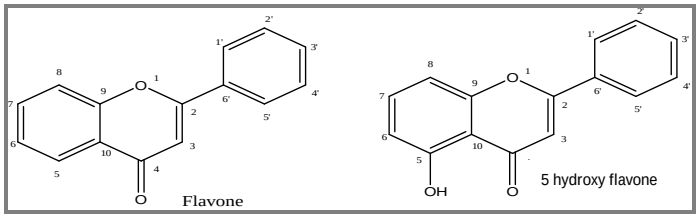
ABSTRACT

Plant metabolites like phytosterols, flavonoids, triterpenoides and alkaloids are very important for many pharmacological activities. Aim of the present study was to identify and characterize the plant metabolites present in Boswellia serrata Roxb leaves. For the isolation of the compounds from the leaves of Boswellia serrata Roxb, powder of dried leaves of the plant was subjected to cold maceration with chloroform as solvent and for column chromatography. Two compounds, compound-1 and compound-2 were identified. These compounds were found to be flavone and 5-hydroxy flavone respectively. These compounds were identified by their chemical tests, RF values, elemental analysis, melting point, IR, ^{1}HNMR, ^{13}CNMR, UV and mass spectroscopy.

Graphical Abstract



Structures of Flavone and 5-hydroxy flavone.

Keywords: *Boswellia serrata* Roxb. plant metabolites, Flavone, Cold maceration.

INTRODUCTION

Boswellia serrata is Indian frankincense tree. This tree is commonly found in Oman, Yemen, West Asia, Southern Arabia, South Africa and many parts of India. In India, it is found in Madhya Pradesh, Bihar, Orissa, Western Himalaya, Rajasthan, Gujarat and Maharashtra [**1, 2**]. Its English word comes from old French frankincense (i.e. high quality incense) and it is used in perfumes and incenses [**3**]. A Sanskrit name "*Gajabhakshya*" sometimes used for *Boswellia* which suggest that elephants enjoy this herb as their diet [**4, 5**]. *Boswellia serrata* Roxb. or salaiguggal contains 20-23% gum, 8-9% essential oil and about 50% resin [**6, 7**].

MATERIALS AND METHODS

Preparation of Plant Material: Green and fresh leaves of *Boswellia serrata* Roxb. were collected from Laling forest in Dhule district (MS); India in the month of November 2014. The plant was taxonomically identified and authenticated by Professor Dr.S. R. Kshirsagar, Taxonomist, Department of Botany, S.S.V.P.S's L.K. Dr.P.R. Ghogrey Science College, Dhule (MS). 3 kg powder of dried leaves was subjected to cold maceration with chloroform at room temperature [**8**].

Isolation and Purification of compounds: A small quantity of chloroform extract was dissolved in chloroform. This solution was spotted on TLC plate. Readymade precoated TLC plates (Merck) of silica gel 60 F 254 were used for spotting. Analytical grade chemicals and reagents were used for TLC. After checking different solvent systems, chloroform:ethylacetate:formic acid in the proportion of 6:3:1 was used as solvent system. Eight gram of chloroform extract was subjected to column chromatography on silica gel (60-120 mesh size) with gradient elution using chloroform:ethyl acetate:formic acid [**14, 15**].

Two fractions showing single spots with clear resolution were subjected to PTLC and the isolated pure compounds were named as compound-1 and compound-2 respectively.

Preliminary Phytochemical test for flavonoids: Both the compounds, compound-1 and compound-2 were subjected to Shinoda, Zinc hydrochloride and alkaline reagent test. These tests showed presence of flavonoids in both the compounds [**8, 9**].

Spectroscopic Characterization: Structures of compound-1 and compound-2 were elucidated using different spectroscopic techniques like UV, IR, ^{1}HNMR, ^{13}CNMR and mass. The UV spectra was recorded on Cary 60 UV-Vis (Agilent Technology) and the IR spectrum was recorded on FT-IR spectrum Two (Perkin Elmer, USA)at SIPS, Sandip Foundation, Nashik(India).The ^{1}HNMR and ^{13}CNMR spectra were recorded on a Bruker Avance (II)400 M Hz. at SAIF, Panjab University, Chandigarh(India). The ^{1}H- NMR and ^{13}CNMR spectra were recorded using $CDCl_3$ as solvent. Mass spectrum was recorded on Waters Micromass Q-TOF micro at SAIF, Panjab University, Chandigarh (India).

RESULTS AND DISCUSSION

Spectroscopic data of compound-1: White powder, **m.p:** 98-100°C, **Yield:**87 mg, **CHN Analysis:** Found: C = 81.187%, H = 4.633%,(calc. for $C_{15}H_{10}O_2$, C = 81.07 %, H = 4.54 %, O = 14.4 %), **UV:** 304 nm, **Mass:** 223.45, **RF:** 0.78, **IR**(KBr)**:** 2853.37 cm^{-1}, 1712.43 cm^{-1}, 1608.11 cm^{-1}, 1382.81 cm^{-1}, 1254.77 cm^{-1}, 1076.78 cm^{-1}, 953.85 cm^{-1}, **^{1}HNMR**(400 MHz, $CDCl_3$)**:** δ 6.8148 (s, H-3), δ 8.2110 (m, H-5), δ 7.4202 (d, H-6), δ 7.6838 (d, H-7), δ 7.6621 (d, H-8), δ 7.9033 (m, H-2'), δ 7.5195 (m, H-3'), δ 7.5397 (m, H-4'), δ 7.5195 (m, H-5'), δ 7.9033 (m, H-6'), **^{13}CNMR**(100 MHz, $CDCl_3$)**:**δ 163.38 (C-2) , δ 107.52(C-3), δ 178.44 (C-4), δ 129.05 (C-5), δ 125.24 (C-6), δ 133.81 (C-7), δ 118.11(C-8), δ 156.312 (C-9), δ 123.92 (C-10), δ 131.64 (C-1'), δ 126.27 (C-2'), δ 129.05 (C-3'), δ 131.70 (C-4'), δ 129.05 (C-5'), δ 126.27 (C-6').

Spectroscopic data of compound-2: Yellow crystalline powder, **m.p.:** 174-176°C, **Yield:** 90 mg, **CHN Analysis:** Found: C = 75.160 %, H = 4.394 % (calc. for $C_{15}H_{10}O_3$, C = 75.62 %, H = 4.23 %, O = 20.15 %), **UV:** 246 nm, **Mass:** 239.54 $[M+H]^+$, **RF:** 0.71, **IR**(KBr)**:** 3185.78 cm^{-1} , 2974.45 cm^{-1}, 1711.96 cm^{-1}, 1610.15 cm^{-1}, 1496.49 cm^{-1} , 1395.85 cm^{-1}, 1225.83 cm^{-1} , 1098.69 cm^{-1}, 960.85 cm^{-1}, **^{1}HNMR**(400 MHz, $CDCl_3$)**:** δ 7.1070 (s, H-3), δ 6.8070 (m, H-6), δ 7.6901(dd, H-7), δ 7.2115 (d, H-8), δ 7.9120 (dd, H-2'), δ 7.5687 (m, H-3'), δ 7.6838 (m, H-4'), δ 7.5687(m,H-5'), δ 7.9120 (m, H-6'), δ 12.1670 (s, OH-5), **^{13}CNMR**(100 MHz, $CDCl_3$)**:** δ 163.38 (C-2), δ 107.52(C-3), δ 183.13(C-4), δ 160.38(C-5), δ118.11(C-6), δ 133.81 (C-7), δ 107.52(C-8), δ 156.22 (C-9), δ 118.11 (C-10), δ 131.64(C-1'), δ 126.27 (C-2'), δ 129.05 (C-3'), δ 133.81 (C-4'), δ 129.05 (C-5'), δ 126.27 (C-6')

Structure elucidation

Compound-1: Compound **-1** was isolated as white crystalline powder of melting point 98-100°C. The UV spectra of compound-**1** showed absorption band λ_{max} at 304 nm. Elemental Analysis of compound-**1**found C = 81.187 % and H = 4.633 %calculated for $C_{15}H_{10}O_2$.The molecular formula ofcompound-**1** was determined to be $C_{15}H_{10}O_2$ by elemental analysis which corresponds to molecular weight 222.4 gm. Mass spectrumof compound-1 showed the peak at m/z 223.45 $[M]^+$ which deduced the molecular formula $C_{15}H_{10}O_2$. Ion peaks were also observed at 150.46, 224.10, 245.43, 246.15 and 247.15

The IR spectrum of compound-**1** showed intense peak at 2980.71 cm^{-1} due to presence of aromatic C-H stretching frequencies. C-H bending vibrations appear at 953 cm^{-1}. The most intense peak absorbed at 1712.43 cm^{-1} showed stretching frequency due to presence of carbonyl (>C=0) group. Peak observed at 1608.11 cm^{-1} due to presence of $H_2C = CH_2$ structure. In IR the absorption band at 1254.77 cm^{-1} showed due to presence of C-O-C structure. The ^{1}HNMR spectrum of compound-**1** showed signals for nine aromatic protons and one acyclic proton. A signal corresponding to H-3 is due to cyclic unsaturated ketone system. The ^{1}H NMR spectrum displayed sharp aromatic protons signals at δ 7.4302 (d, H-8), δ 7.6838 (d, H-7), δ 7.6621 (d, H-8) which suggested a flavone skeleton. The ^{13}CNMR spectrum of compound-**1** showed the presence of 15 carbon signals. The signal corresponding at δ 178.44 is the most downfield peak; which was assigned to ketone group (C-4). The signal δ 163.38 is next downfield signal corresponding to C-2 i.e. C-O structure. The ^{13}CNMR already shows 15 carbon atoms in the molecule those at C-2' and C-6' are equivalent as are those at C-3' and C-5' due to free rotation around the C-2/C-1' bond in accordance with expected number of carbon signal. This permitted the tentative assignment of most intense signal at δ126.27 and δ129.05 ppm. Some other tentative assignments could also be made. The carbonyl carbon (C-4) was observed to the lowest field signal at δ178.44 ppm. C-2 and/or C-9 at δ163.38 and/or δ156.322, C-10 and/or C-1' at δ131.64 and/or δ123.92 ppm, and C-3 to the highest field signal δ107.52 ppm since it is confirmed that sp^2 C-H group is present. By considering phytochemical and spectroscopic assignments following structure is deduced for the compound-**1** which is in good agreement with structure of flavone (Figure 1) [**10, 11**].

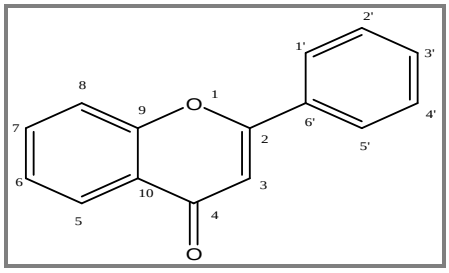


Figure 1. Structure of Flavone.

Compound-2: Compound-2 was isolated as yellow crystalline powder of melting point 174-176°C. The UV spectra of compound-**2** showed absorption band λ_{max} at 246 nm. Elemental Analysis of compound-**2** found C= 75.160 % and H = 4.394 %. Calculated for $C_{15}H_{10}O_3$ .The molecular formula of compound-**2** was determined to be $C_{15}H_{10}O_3$ by elemental analysis which corresponds to molecular weight 238.24 gm. Mass spectrum of compound-**2** showed base peak at 239.54 $[M+H]^+$ which deduced the molecular formula $C_{15}H_{10}O_3$. Ion peaks were also observed at 150.46, 224.10. The IR spectrum of compound-**2** showed broad peak at 3185 cm^{-1} which is characteristic for O-H stretching suggesting presence of hydroxyl group which was substantiated by chemical identification tests. The absorption band at 2974.45 cm^{-1} corresponds to CH_2 stretching. In IR spectrum the intense peak at 1711.96 cm^{-1} showed stretching frequency due to presence of carbonyl (>C=O) group.1610.15 cm^{-1}

stretching frequency showed presence of CH = CH group. The absorption band at 1225.82 cm^{-1} is due to presence of C-O-C stretching.

The ^{1}HNMR spectrum of compound-**2** showed signals for eight aromatic protons, one allylic proton and one hydroxyl proton. The ^{1}HNMR spectrum of compound-**2** showed presence of aromatic proton signal of δ H in range between δ 6.81ppm -δ 7.9120 ppm. The ^{1}HNMR signal at δ12.6704 ppm was observed due to presence of C-OH group i.e. Hydroxyl proton. The ^{1}H NMR signal at δ 6.807 (d) and δ 7.2115 (d) ppm showed due to presence of H-6 and H-8 of two protons, H-6 can show cross peak with hydroxyl protons so that δ 6.81 ppm signal should be for H-6 and not for H-8. As a result H-8 can be assigned δ 7.2115 ppm.

The ^{13}C NMR spectrum of compound-**2** showed the presence of 15 carbon signals. The signal corresponding at δ 180.13 ppm is the most downfield peak which was due to presence of (>C = O) ketonic group. The ^{13}CNMR signal at δ 160.38 is next downfield signal corresponding to group C-O structure. The signal at δ 160 ppm is due to presence of C-OH group at C-5, the downfield signal is due to electron withdrawing group (-OH) is present at carbon atom .The singlet peak absorbed at δ 118.11 ppm was assigned to C-10 because C-6 and C-8 could not be a singlet. Two ^{13}C peaks at δ 118.11 and δ 107.52 ppm observed were attached to the ^{1}H NMR peaks at δ 6.8070 and δ 7.2115 ppm respectively. ^{13}CNMR spectrum showed the presence of 15 carbon signals but out of 15 carbon atoms in the isolated molecule, those at C-2$^{'}$ and C-6$^{'}$ are equivalent as are those at C-3$^{'}$ and C-5$^{'}$ due to free rotation around the C-2/C-1$^{'}$ band in accordance with expected by considering the phytochemical and spectroscopic assignments following structure is deduced for the compound-**2** which is in good agreement with the structure of 5-hydroxy flavone (Figure 2)[**12-19**].

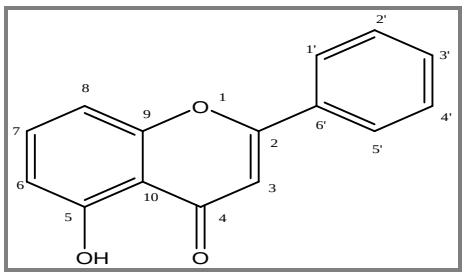


Figure 2. Structure of 5- hydroxy flavone.

APPLICATION

Flavonoids have beneficial effects on health. Some of them work on intracellular replication of viruses while others inhibit the infectious properties of the viruses. They can inhibit angiogenesis and also can inhibit cytosolic and membranal tyrosine kinase. Some of the flavonoids show considerable cytotoxicity at higher concentration.

CONCLUSION

Isolation of phytoconstituents present in *Boswellia serrata* leaves extract by cold maceration method using chloroform as solvent [20a-e] resulted in to two compounds (flavone) and (5-hydroxy flavone) respectively. These compounds were analyzed by using physical, chemical and spectroscopic analysis. These active medicaments can be studied further in future for their biological activities.

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