International Journal of Advanced Research and Development
www.multistudiesjournal.com
ISSN: 2455-4030
Received: 21-07-2024, Accepted: 20-08-2024, Published: 04-09-2024
Volume 9, Issue 5, 2024, Page No. 3-6

Chemical constituents from petroleum ether extract of leaves of *Butea monosperma* Roxb. and their uses in antimicrobial and antifungal activity

Neelesh Kumar Pandey[1], Ravi Prakash[1], RB Singh[2]
[1] Department of Chemistry, B.S.A. College, Mathura, Uttar Pradesh, India
[2] Scientist, Department of Zoology, School of Life Science, Dr. Bhimrao Ambedkar University, Khandari Campus, Agra, Uttar Pradesh, India

Abstract
A comprehensive phytochemical investigation of the petroleum ether extract of leaves of *Butea monosperma* Roxb. Resulted in the isolation and identification of two known compounds β-carotene and stigmasterol. These two compounds were isolated from leaves of plant for the first time. Their structures were identified by physical, chemical, elemental analysis and spectroscopic methods including U.V., FTIR, MS, ^{1}H NMR, ^{13}C NMR. These compounds also showed significant antibacterial and antifungal activity.

Keywords: Butea monosperma, fabaceae, Beta carotene, stigmasterol

Introduction

Literature on traditional medicinal is very merge. In India plenty of plant being used as drug due to their medicinal properties. The plant kingdom still holds many species of plant contains substance of medicinal value which are yet to be discovered [1, 2]. One of them is leaves of *Butea monosperma Roxb*. It is medium sized tree with 20-40 feet height belonging to the family Fabeacae. It is found in mountain region of India, Burma and few Asian countries [3] this plant is extensively used in India to treat various diseases. The flowers are used in the treatment of hepatic disorders, viral hepatitis, diarrhea, anticonvulsive agent and tonic [4, 5, 6] the roots are useful in treatment of night blindness, piles, ulcers, tumer [7] the gum is powerful astringent. The stems bark possesses antifungal activity. Phytochemical investigation showed the presence of different classes of compounds e.g. flavonoid [8, 9] from flowers, sterols [10] from stem bark. In continuation of our studies on medicinal plants the literature survey reveals that very less work is done on leaves of Butea monosperma. In present paper we are reporting the isolation characterization of two known compounds and their antibacterial and antifungal activity.

Materials and methods

General experimental procedures: Melting points were measured on melting point apparatus. Optical rotations were determined in $CHCl_3$ solutions (Equiptronics digital polarimeter, EQ- 801).UV spectra were recorded in $CHCl_3$ solution. FTIR was taken in KBr (MAGANA 550, range 4000cm to 50 cm^{-1} Make- Nicolet Instruments -1 Corporation,USA), ^{1}H-NMR and ^{13}C-NMR spectra were taken on 300 MHz and 75 MHz respectively. (Mercury plus 300 MHz Make Varian USA) Mass spectra were taken on 410 Prostar Binary LC with 500 MS IT PDA Detectors. (Make Varian Inc, USA having specification of direct Infusion Mass with ESI & APCI Negative & Positive mode ionization, mass ranging from 50 to 2000 m/e). Elemental analysis were done on Flash EA 1112 series (Make-Thermo finnigan, Italy).

Extraction and isolation: Accurately weighed 50 gm of leaves powder was loaded in Soxhlet's extractor and defatted with petroleum ether (60-80° C) in 20 batches (50-60 cycles in each batch). The progress of the extraction was evaluated by applying spot of extract on thin layer chromatography plate. The thin layer chromatography was performed using silica gel plate and the plate was visualized in UV-chamber followed by iodine chamber. The extract was filtered and concentrated by rotary evaporator and finally dried at very low pressure obtained dark greenish black semisolid mass. The petroleum ether extract confirms the presence of sterols and triterpenes [11]. The petroleum ether extract of leaves of *Butea monosperma* was saponified using IM alcoholic KOH, to remove fatty material and then subsequently picked up in ether and solvent was evaporated to yield 7 gm of unsaponified matter. A small portion of unsaponified matter of petroleum ether extract was dissolved in CHCl, and solution was spotted on TLC plates. Then TLC plates were run by specific solvent system and were viewed individually under UV light and Vanillin H_2SO_4 reagent.[12] Through several pilot reading, it was found that the compounds of unsaponifiable matter were separated by the solvent system of benzene methanol (9:1). Column chromatography of unsaponifiable matter was conducted using silica gel (mesh 60-120) the silica gel activated at 110° C for 60 minute then it was mixed with mobile phase and slurry was prepared. The column was filled with slurry of silica gel without formation of any air bubble. The column was then allowed to stabilize for overnight. The 3 gram of unsaponiable matter vigorously mixed with silica gel and solvent. The solvent was then evaporated to free flowing material. This material was charged in column. The column was run using benzene methanol (9:1) by gradient elution technicque. 130 fractions each of 5ml were collected. TLC was used to monitor each fraction. Fraction shows similar separation (i.e. same Rf value) on TLC plates were mixed. Fractions show two or more spots that were further purified by recolumn. However fractions showing inseparable mixture of compound were rejected. Further purification is carried out by preparative thin layer chromatography bands were identified, scraped

and extract with chloroform. Two pure compounds were isolated by column, recolumn, and by preparative thin layer chromatography.

Compound characterization

Beta carotene: Red purple crystal; melting point is 182-184 C. UV ($CHCl_3$) λ_{max} at 470 and 493 nm. The IR absorption peaks at 2950 cm and 2915 cm^{-1} corresponds to C-H vibration.1618 cm^{-1} and 1558 cm^{-1} corresponds to >C=C<vibrations.

^{13}C NMR (75 MHz, $CDCI_3$): δ29.0 (s, C-1), 31.6 (t, C- 4), 34.3 (t, C-3), 39.67(t, C-2), 125.1 (d, C-15), 126.7 (d, C-14), 129.4 (s, C-13), 130.0 (d, C-12), 130.9 (d, C-11), 132.5 (d, C-10), 136.0 (s, C-9), 136.5 (d, C-8), 137.3 (d, C-7), 137.8 (s, C-6), 137.9 (s,C-5).

^{1}H NMR (300 MHz CDC13) δ: 0.98-1.06 (s, 12H), 1.54-1.42 (m,4H), 1.66-1.56 (m,5H), 1.74-1.68 (m,5H) 1.74-1.68 (m,5H), 1.92-1.88 (m,1H), 2.0-1.94 (m,10H), 2.08-2.02 (m,5H),6.4-6.04 (m,10H), 6.69-6.50 (m,4H).

MS Spectroscopy showed the molecular ion peak at 537.4 corresponds to molecular formula $C_{40}H_{56}$. Ion peak also observed at m/z 457, 279, 244, 199, 159,

Stigmasterol: It is white crystalline substance; melting point 171-172 °C. UV Spectroscopy. UV ($CHCl_3$) λmax at 257 nm. In the IR of compound 2 an intensively broad band at 3345 cm^{-1} and moderately intense band at 1192 cm^{-1} and 627 cm^{-1} were observed for -O-H band vibration of the hydroxyl group. The out of plane -C-H vibration of the unsaturated part was observed at 890 cm.The corresponding >C=C< vibrations was observed at 1653 cm^{-1} as weakly intense band. The intense band at 2934 cm^{-1} and medium intensity band at 1458 cm^{-1} noticed the stretching and bending vibration of methyl part. The vibration of the methylenic part was shown by band at 2866 cm^{-1} and medium band at 1381 cm^{-1}. The moderate intense band at 738 cm was attributed to rocking movement of methylene part, the corresponding C-C vibration was shown weak intense band 1022-75 cm^{-1}.

^{13}C NMR (75 MHz, $CDCI_3$): δ 12.1 (q, C-18), 12.2 (q, C-29), 12.3 (q. C-19), 19.0 (q, C-28), 19.4 (q, C-26), 21.1 (q C-27), 21.2 (q, C-21), 21.3 (t, C-11), 24.4 (t, C- 12), 25.4 (t, C-2), 25.5 (t, C-15), 25.7 (t, C-16), 29.0 (d, C-8), 31.9 (t, C-7), 36.5 (t, C-1), 39.7 (d, C-20), 40.5 (d, C-13), 42.2 (d, C-24), 42.3 (d, C-25), 50.2 (d, C-9), 51.0 (t, C-4), 51.3 (s, C-10), 55.9 (d, C-17), 56.9 (d, C-14), 71.7 (t, C-3), 121.7 (d, C-6), 129.3 (s, C-5), 138.3 (d, C-23), 140.8 (d, C-22).

^{1}H NMR(300 MHz, $CDCl_2$): δ 1.2 (2H, t, H-1), 0.98 (2H, m, H-2), 1.2 (2H, t, H-1), 3.6 (1H, m, H-3), 1.30 (2H, d, H-4), 5.38 (1H, t, H-6), 5.1 (1H, m, H-14), 4.94 (1H, m, H-17), 1.06 (3H, s, H-18), 1.20 (3H, s, H-19), 3.6 (IH, m, H-20), 0.9 (3H, s, H-21), 5.33 (1H, t, H- 22), 5.35 (1H, t, H-23), 2.33 (1H, m, H-24), 2.1 (1H, m, H-25), 0.88 or 1.88 (6H, d, H-26,27), 2.33 (2H, m, H-28), 0.84 (3H, t, H-29), 7.25 (1H, s, OH)

MS spectroscopy showed the molecular ion peak at 412.3corresponds to molecular formula $C_{29}H_{48}O$. Ion peak also observed at m/z 391, 366, 269, 254, 229, 215, 189, 161, 153.

Biological evaluation:

The isolated compounds were preserved in labeled sterile screw capped bottles at-20 °C. Antibacterial and antifungal activity assays were performed by the modified disc diffusion method [13, 14] the bacterial and fungal strains were used obtained from National Collection of Industrial Microorganism (NCIM), Pune, India. Petri dishes (5 cm diameter) were filled up to a depth of 3-4 mm with sterile nutrient agar (Hi-Media) for bacteria and Meat extract, Glucose yeast extract, Peptone medium i.e. yeast for fungi. A sterile Whatman filter paper disc of 6 mm diameter preloaded with 100 mcg of target compound in DMSO was placed in the centre of the nutrient agar plates of bacteria and MAYP plates of fungi. Four plugs of bacterial inoculums and fungal inoculums were placed upside down at the quarter circle points 20 mm radius around the drug loaded disc in the Petri dishes. Blank control disc were treated with DMSO, Chloramphenicol for bacteria and Nystatin for fungi was used as standard. The stringent aseptic conditions were maintained during microorganism inoculation and the plates were labeled. The Petri plates were incubated at 37±1°C for 24 hours for antibacterial screening and at 25° C for 2-7 days for antifungal screening. The diameter of zone of inhibition of each disk was recorded. The results of antibacterial activity were tabulated in Table-l. The results of antifungal activity were tabulated in Table-1.

Table 1: Antibacterial screening

Compound	Zone of inhibition			
	E. Coli	*P. aeruinousa*	*B.Substails*	*S.aureus*
1	14.56	15.63	18.23	17.23
2	18.23	16.20	18.68	19.20
Chloramphenicol[b]	25.30	30.94	20.52	20.49

[a]Zone of inhibition in mm, diameter in mm calculated by venire caliper.

[b]Concentration of standrad drug, chloramphenicol was set to 10 mcg/disc.

Table 2: Antifungal screening

Compound	Zone of inhibition	
	A. niger	*C. albicans*
1	10.12	9.88
2	11.10	8.23
Nyastatin	9.67	9.53

[a]Zone of inhibition in mm, diameter in mm calculated by venire caliper.

[b]Concentration of standrad drug, Nyastatin was set to 100 U/disc.

Results and discussion

The dried powder [50 gm] of the leaves of Butea monosperma was extracted with petroleum ether (boiling point 60-80°C) in soxhlet extractor. Removal of solvent from the petroleum ether extract gave a mass of 1.5 gm. The process was repeated for 10 times to get sufficient extract ie. 15 gm. The petroleum ether extract confirmed the presence of sterols and triterpenes qualitatively by phytochemical test. The extract was then saponified with alcoholic KOH, to remove fatty material. The compounds present in unsaponifiable matter were separated by column chromatography, recolumn chromatography and preparative thin layer chromatography and purified by chloroform. Two compounds were isolated.

Compound 1 was isolated as red purple crystals; melting point is 182-184° C. It gave positive Carr- Price test [15] 85% H_2SO_4 (blue color) and conc. HCl phenol solution (blue color) these tests are characteristics for carotenes. Carotenoids have basic structure made up of isoprene units which are joined end to end to give a conjugated chain which is common to all carotenoids. The long conjugated chain is responsible for the orange color of compound 1.The UV spectra of compound I in chloroform showed characteristics absorption band (λmax) at 460 and 493 nm. Mass spectra of compound 1 showed base peak m/z at 537, which deduced the molecular formula $C_{40}H_{56}$. Elemental analysis results for compound 1 (Found: C, 88.87; H, 9.99 Calc.for $C_{40}H_{56}$: C, 89.49; H, 10.50%).

The IR spectrum of compound 1 displayed at 2950 cm^{-1} and 2915 cm^{-1} (C-H) vibrations and 1618 and 1558 cm^{-1} (>C=C<) vibration.

The ^{1}H NMR spectrum of compound 1 shows strong singlet for 15 H. As it shows positive test for carotenoids, they have a basic structure made up of isoprene unit. The two centre isoprene units are joined differently to others head to head so that chain has centre of symmetry. Therefore structure of compound 1 is symmetrical. The structure is divided in to two equal halves. There are five CH_3 group in each half. As these CH_3 groups are attached to tertiary carbon atom all of them gave a strong singlet in ^{1}H NMR spectrum. Among these CH_3 group, the CH_3 group attached to carbon number 1 showed a singlet at 0.9 8. While another equivalent methyl groups that are attached to carbon number 5,9,13, also showed singlet. Expected theoretical value for this singlet is 0.9 8 but CH_3 is attached to a double bonded carbon, the 8 value shifts to downfield and peak appears at 1.1δ.

All the hydrogen atoms shown in the structure except hydrogen at C-11 and C-11'showed doublet in the spectra as well as they are Trans coupled. The expected Trans coupling frequency is 10-12 Hz. Doublet expected to appear at between 1.4 to 1.5 8 values. The C-11 and C-11 hydrogens showed triplet because of adjacent two hydrogens on adjacent carbon atoms. Being a symmetrical structure these two hydrogens are equivalent and showed a triplet at 1.92 δ.

Compound 2 was isolated as white crystals; having melting point is171-172 °C. It gave positive test for alcohol as well as Salkowski reaction (reddish color) and Libermann-buchard reaction (violet blue and finally green) for steroids [16, 17] [α] D^{23}-51° in (c 0.01, $CHCl_3$) UV Spectroscopy. It showed UV max. At 257 nm. MS spectroscopy showed the molecular ion peak at 412.3, which corresponds to molecular formula C29H48O. Ion peak also observed at m/z 391, 366, 269, 254, 229, 215, 189, 161, 153. The molecular formula of compound 2 was also supported by elemental analysis. Elemental analysis results for compound 2 (Found: C, 84.31; H, 11.72. Calc.for C_{29} $H_{48}O$: C, 84.40; H, 11.72%).

In the IR spectrum of compound 2 an intensively broad band at 3345 cm^{-1} and moderately intense band at 1192 cm^{-1} and 627 cm^{-1} were observed for -O-H band vibration of the hydroxyl group. The out of plane -C- H vibration of the unsaturated part was observed at 890 cm^{-1}. The corresponding >C=C< vibrations was observed at 1653 cm as weakly intense band. The intense band at 2934 cm^{-1} and medium intensity band at 1458 cm^{-1} noticed the stretching and bending vibration of methyl part. The vibration of the methylenic part was shown by band at 2866 cm^{-1} and medium band at 1381 cm. The moderate intense band at 738 cm was attributed to rocking movement of methylene part, the corresponding-C-C-vibrations showed weak intense band at 1022-75 cm^{-1}187 The ^{1}H NMR spectrum of compound 2 showed the olefin protons appeared as triplet at 5.38 8.

Another olefin proton H-22 and H-23 appeared as triplet at 5.33 and 5.35 δ respectively. It also showed strong singlet of IH for OH at 7.25 8. It showed singlet for two tertiary CH, group of carbon number 18, 19 at 0.7 8 and 21 at 0.9 8. This compound also showed doublet for two equivalent CH, group of carbon number 26 and 27 at 0.88 8.

The antibacterial and antifungal activity of compounds 1 and 2 were shown in Tablo 1 and 2. The compound 1 and 2 showed significant antibacterial and antifungal activity. The compound 1 showed maximum inhibition zone for E.coli. The compound 2 showed maximum inhibition zone for P.aeruginosa. Compound 1 and 2 showed more antifungal activity as compared to standard.

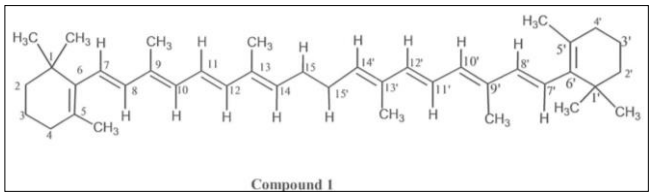

Fig 1

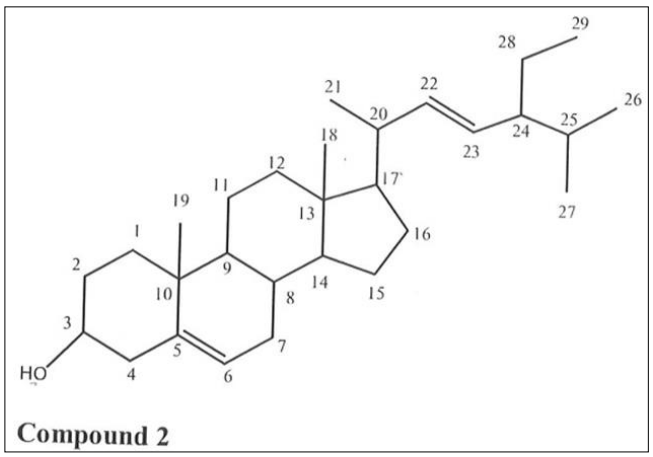

Fig 2

References

1. Rajput AP, Yadav SS,2000:13(2):161-167.
2. Ambask SP. The Wealth of India. A dictionary of Indian Raw Material and Industrial Products. Publication and Information Directorate CSIR; New Delhi. India,1988:2(B)341-347.
3. Kasture VS, Chopade CT, Deshmukh YK. Journal of Ethanopharmacology,2000:200(71):65-75.
4. Mishra M, Shukla Y, Sushil Kumar, Phytochemistry,2000:54(8):835-838.
5. Nadkarni AK, Indian Materia. Bombay Popular Prakashan; Mumbai, 1982, 222-224.
6. Agrawal AK, Tripathi DM, Sahai R, Gupta N. Journal of Ethanopharmacology,1997:56:223-236.
7. Gupta SR, Ravindranathan B, Sheshadrai TR, Phytochemistry, 9, 2231-2235.

8. Wagner H, Geyer B, Fiebig M, Kiso Y, Hakino H, Planta Med,1986:52(2):77-79.
9. Ratnayake Bandara BM, Savitri Kumar N, Swarna Samarnayake KM. Journal. of Ethnopharmacology,1989:25(1):73-75.
10. Rajput AP, Pal SC, Patil BS. International Journal of Pharmacy and Pharmaceutical Sciences,2011:3(3):189-191.
11. Stahl E, Thin Layer Chromatography: A Laboratory Handbook. 2nd Edn, Springer, New York (1969).
12. Vivek YS, Seung RK, Hak RK, Sun CK, Ind. Crops Production,2008:27:136-141.
13. Zhijun S, Weiquan C, Xiaoying D, Heng Z, Lijing L, Hanhong X, Phytochemistry.,2011:72:490-494.
14. Ewans, W.C., Treas and Evans Pharmacognocy. 15th ediction: Sounders. An imprint of Elsevier Science, 2002, 325-326.
15. Harborne JB. Phytochemical Methods Techniques of Plant Analysis. Chapman and Hall; London, 1996, 4-7.
16. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. Nirali Prakashan Pune., 2007, 149-156.
17. Jain PS, Bari SB. Asian J. Plant Science,2010:9:163-167.
18. Ian R, Goad JL, Clague ADH, Mulhetiam LJ. Phytochemistry,1975:15:195-200.