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Research Article

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New terpenoides and Steroids from Syzygium cumini Barks

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ABSTRACT

A new pyrazole like compound namely, (E)-4-((2-hydroxy-1-phenylethylidene) amino)-1, 5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (HEDP) and its Ni (II), Fe (III) and Zn (II) metallic complexes have been synthesized. These testers had characterized from side to side spectral and orderly ways and means. At liberty ligand behaves as tridentate with hydroxyl, carbonyl and imine moiety were co-ordination sites. The FT-IR stretching frequency for the above co-ordination sites were varied after co-ordination with Ni (II), Fe (III) and Zn (II) metallic salts. The octahedral geometry of the metallic multiplexes were confirmed by spectral techniques such by way of UV-Vis, FT-IR, 1H-NMR, 13C-NMR, ESI-MS moreover EPR. Living doings of the ligand and over its multiplexes devour be located verified by anti-oxidant reading, anti-microbial strains such as Escherichia - coli, Staphylococcus aureus, Candida albicans (227) and Penicillium chrysogenum or P.notatum. Metallic multiplexes took larger living events than free HEDP. The computational analysis like biological activity prediction, quantitative prediction of anti-target interaction, cell line cytotoxicity and docking of the samples were done with online software and commercial docking software.

Keywords: Syzygium cumini (Bark); Terpenoids; Steroids; Benzene extract; Antimicrobial; Antifungal activity

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INTRODUCTION

Syzygium cumini belongs to Myrtaceae family has commonly known as Malabar plum, Java plum, black plum, or jambolan. It has one of the widely used medicinal plants in the treatment of various diseases in particular diabetes. *S. cumini* showed various phytoconstituents such as tannins, alkaloids, steroids, flavonoids, terpenoids, fatty acids, phenols, minerals, carbohydrates and vitamins. Its pharmacological actions like hypoglycaemic, analgesic, anti-inflammatory, anti-plaque, antimicrobial, anti-diarrhoeal, antioxidant, gastro-protective and astringent to bowels proven on animal models. Different parts of the jambolan were also reported for its antioxidant, anti-inflammatory, neuropsychopharmacological, antifungal, antibacterial, antiviral, nitric oxide scavenging, anticancer, free radical

scavenging, anti-diarrheal, anti-fertility, anorexigenic, gastroprotective and anti-ulcerogenic and radioprotective activities. In view of its wide spectrum activities, benzene extract of barks furnished a new terpenoides and steroids [1].

MATERIALS AND METHODS

General Experimental Procedure Melting Points (MP) are uncorrected. 1H NMR spectra were measured on a solution of the glycoside 1 (30 mg) in D_2O and 2, 3, 4 and 5 (28 mg) in CDC_{l3} at ambient temperature. The high resolution 1D and 2D NMR spectra ($^1H^1H$COSY, HMQC and HMBC) and ^{13}C-NMR spectral analyses were performed using a JEOL-JNM-300 and 75 MHz spectrometer. All chemical shifts are given in ppm and (tetra methyl silane) TMS was used as an internal standard (Figure 1). The degree of protonation on carbon (CH_3, CH_2 and CH) was determined by DEPT (90, 135) experiments. Conventional pulse sequences were used for COSY, HMQC and HMBC. The Thin layer chromatography was performed using silica gel G and spots were visualized by exposure to iodine vapors or by spraying with H_2SO_4 vanillin solution followed by heating at 105ºC for 5 min.

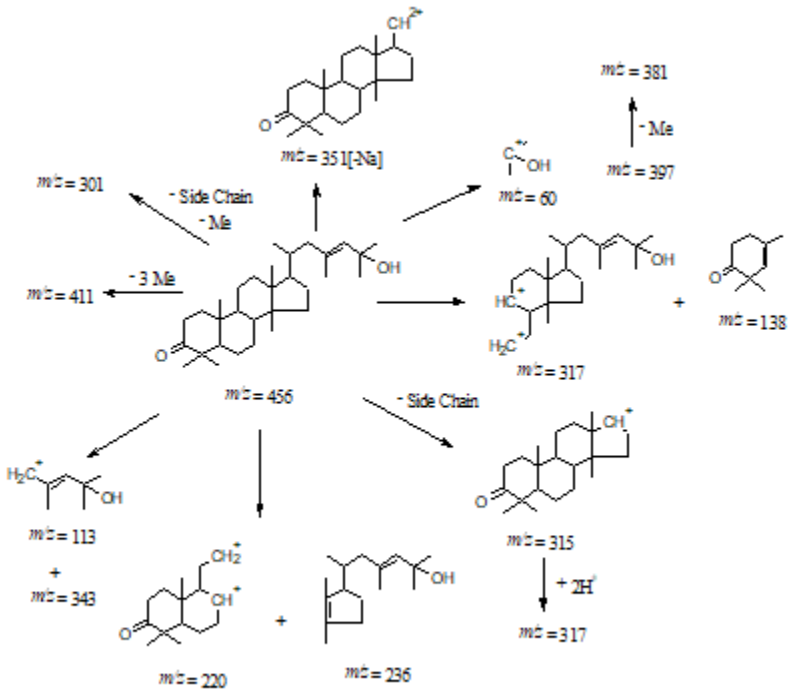

Figure 1: Fragmentation patterns proposed

The Barks (10 kg) of S. cumini were collected from the local medicinal market of Ujjain city and were authenticated by the authorities at EIMPS, Vikram University, Ujjain.

Extraction and isolation of the constituents

Ten kilograms of shade-dried, cleaned and coarse-powdered bark were extracted by n-hexane, benzene, benzene: acetone and alcohol serially each for 72-75 h in soxhlet extractor. Benzene extract was concentrated to dryness under reduced pressure by rotary film evaporator to afford a dark brown syrupy residue (370 mg). The dried sample was fractionated on normal phase silica gel column chromatography (1000 cm × 25 cm) by using gradient elution with different solvent mixtures in their increasing order of polarity. A dried portion of the 50% benzene:MeOH (145 mg) elute was subjected to repeated chromatography on silica gel, using a discontinuous gradient from 1:1 benzene : EtOAc to 1:1 benzene : methanol. Fractions 40-50 (2500 mL) of afforded colourless gummy solid (60 mg), in pure form designated as 1. Hexane and benzene fractions of alcohol extract further separated by rechromatography over alumina grade III and afforded two compounds in pure form designated as 2 and 3 respectively. (Benzene: EtOAc, v/v 9:1) fraction of alcohol extract further rechromatographed over silica gel and eluate benzene and (benzene: ether, 8:2, v/v) gave compound 4 in pure form and 5 in crystalline form, respectively.

RESULTS AND DISCUSSION

The natural compounds were identified mainly by their ^{1}D (^{1}H and ^{13}C NMR), 2D (^{1}H^{1}H COSY), DEPT and mass spectrometry analysis, including comparison with literature data. The ESI-MS and elemental analysis of 1 indicated the molecular ion peak at m/z 442 (M^{+} +Na) suggesting its molecular formula $C_{30}H_{50}O_2$. Its IR spectrum displayed typical absorption bands for hydroxyl group at 3431 cm^{-1} and long chain aliphatic nature of the molecule (2869, 1456, 1376, 1236, 1107, 1044 cm^{-1}). Band at 1686 cm^{-1} showed the presence of unsaturation in the molecule. In its ESI mass spectrum, the regular differences of m/z 189 mass units were observed which indicating that 1 was due to fragment formed as a result of hemolytic cleavage between C_8-C_{14} bonds and C_{12}-C_{13}. The peak at *m/z* 154 was due to fragment formed as a result of hemolytic cleavage between C_5-C_{10} bonds further loss of H_2O molecule from *m/z* 154 results in the formation of peak at *m/z* 136. The important peaks were obtained at *m/z* 411 (M – $2CH_3$), 255, 215, 189, 154, 136, 107, 95 and 82 justified the proposed structure of 1. The fragmentation pattern was given in Figure 2. Complexities of the signals appeared in 1H NMR of these compound contain one proton signal as a doublet near δ 2.24 (J = 10 Hz) due to an

allylic 18- hydrogen. It showed sharp signals for seven tertiary methyl's at carbon no. C-23, C-24, C-25, C-26, C-27 and C-28 appeared as singlets at δ 0.99, 0.99, 1.25, 0.75, 0.93 and 0.82 respectively and doublets for C-29 at δ0.97 gave clear indication of compound to be ursane. A broad singlet at δ1.69, a doublet at δ 4.60 (^{1}H, *J*=1.5 Hz) and quartet at 4.74 (^{1}H, *J*=1.5 Hz) confirmed the presence of exocyclic double bond at 20. The appearance of this exocyclic double bond at C-20(30) as doublet and quartet suggested the compound to be of taraxasten series. The hydroxyl protons resonated at δ 1.92 while carbinolic proton resonated at δ 4.08 and 3.18.The chemical shifts in ^{13}C NMR spectra suggested a close resemblance with 20(30) – ursane. The quaternary carbon (C_{20}) resonated at 152.01 ppm. The olefinic carbon (C_{30}) resonated at 105.0 ppm. In the ^{13}C NMR spectrum of triterpene the peaks at 23.8, 23.2, 21.1, 16.7, 23.6, 28.4 and 17.1 ppm confirmed presence of seven methyls. The carbinolic carbon C-2 and C-3 were appearing at 68.9 and 83.9 ppm. Hence compound 1 is: 2β-3β-Dihydroxyurs-20(30)-ene [2].

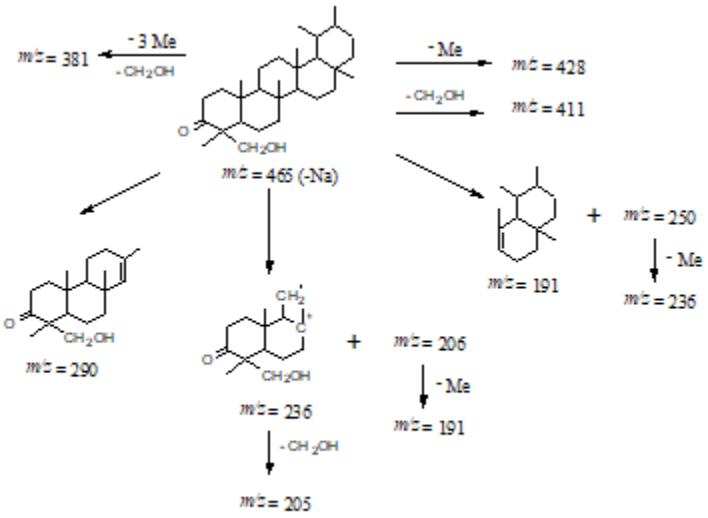

Figure 2: Structure showing the fragmentation patterns proposed

On the basis of above evidences, 1 was identified and characterized as: 2β-3β-Dihydroxyurs-20(30)-ene. IR spectrum of 2 showed typical absorption bands for alcohol and carbonyl groups (3452 cm^{-1}, 1718 cm-1) and Band at 1640 cm^{-1} showed the presence of unsaturation in the cyclic ring. The ESI-mass spectral data and elemental analyses associated to the NMR data of 2 are coherent with the molecular formula $C_{31}H_{52}O_2$. The pattern of the ESI-MS spectrum revealed that 2 was a characteristic fragmentation pattern of a Triterpene. The molecular ion peak appeared at *m/z* 481 (M^+ -Na, -2H^+)

and the molecular formula was found to be $C_{30}H_{50}O_2$. The important peaks were obtained at *m/z* 411 ($M^+ - 3CH_3$), 301 (M^+ - side chain, $-CH_3$) and 317(M^+ - side chain). The peak at *m/z* 236 and 220 was due to fragment formed as a result of hemolytic cleavage between C_8-C_{14} and C_{12}-C_{13} bonds. Other important fragment formed at *m/z* 381, 353, 131, 113, and 60. The fragmentation pattern was given in Figure 3. 1H NMR spectrum showed eight singlet signals representing eight methyl groups at a δ 0.73, 0.95, 1.01, 1.05, 1.13, and 1.26, for C-18, C-30, C-29, C-31, C-19, C-26 and C-27 protons respectively and doublet signal representing one methyl group at δ 0.87 for C-21 protons. The presence of all the methyl groups as singlet supported the fact that all methyl groups in lanostane were attached to quaternary and secondary carbons. Singlet signal appeared at a 5.59 for one olefinic proton at C-24 proton. The presence of eight methyl groups, including one secondary methyl group (δ 0.87, d, 3H, *J* = 6.3 Hz), five quaternary methyl groups (δH 0.73, 1.13 1.01, 0.95, 1.05), and two methyl groups geminal to a quaternary carbon bearing hydroxyl group [δH 1.26 (s, 6H); δ C 30.1, 30.2 and 70.9]. Multiplate signal appeared at a δ 2.40 for C-2 protons coupled with the protons of C-1. One sharp singlet at a δ 1.57 represented protons for C-28 methyl group which was attached on C-23 olifinic carbon. Multiplate at δ 1.78-2.04 showed rest of the protons presented in molecule. Above evidence suggests 2 to be a tetracyclic triterpene.

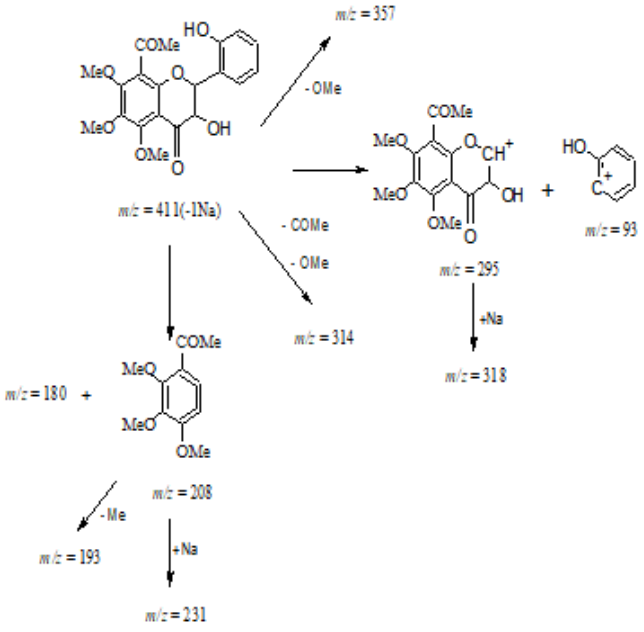

Figure 3: Structure showing the fragmentation patterns proposed

The chemical shifts in C NMR spectra suggested that the molecule is triterpene and the peaks at 18.06, 22.4, 30.1, 30.2, 14.6, 15.8, 28.4, ppm confirmed presence of seven methyls for C-18, C-19, C-26, C-27, C-28, C-29 and C-30 respectively. The peak at 18.5ppm confirmed C-21 methyl. The carbonyl carbon C-3 was appearing at 213.16 ppm. The unsaturated carbons resonated at 150.04 and 110.0 ppm for C-23 and C-24 carbons. Thus on the basis of above spectral evidences, 2 was characterized as Lanosta-23-methyl-23-ene-25-ol-3-one, it is a new compound being reported for the first time by us. IR spectrum of 3 showed absorption bands for alcohol group (3421 cm^{-1}) and for the long chain aliphatic nature of the molecule (2930, 2360, 1261, 1118 and 618 cm^{-1}). Weak band at 1601 cm^{-1} showed the presence of unsaturation in the molecule. The ESI-MS spectrum and combined $^{1}H/^{13}C$ NMR studies of 3 suggested the molecular formula as $C_{29}H_{50}O_2$. In its ESI-MS spectrum, molecular ion peak obtained at m/z 453 (-Na) suggesting molecular formula $C_{29}H_{50}O_2$, to be a steroidal molecule. The separation of most of the important peaks were at m/z 413 (M- H_2O showed characteristic fragmentation of a steroid), and indicated that it was a steroidal compound. The characteristic pattern of steroidal molecule -397 (M^+-H_2O, -CH_3), 289 (M^+-side chain), 257 (M^+-side chain+ H_2O+ -CH_3). The other fragments were obtained at *m/z* 414 (M^+-CH_3), 381, 301, 245, 141and114 were in agreement with the proposed structure. The ^{1}H NMR spectrum showed singlets at δ 0.68 and 1.01 for two angular methyl groups at C-18 and C-19. A doublet at δ 0.93 was assigned to C-21 methyl group (Figure 4) [3].

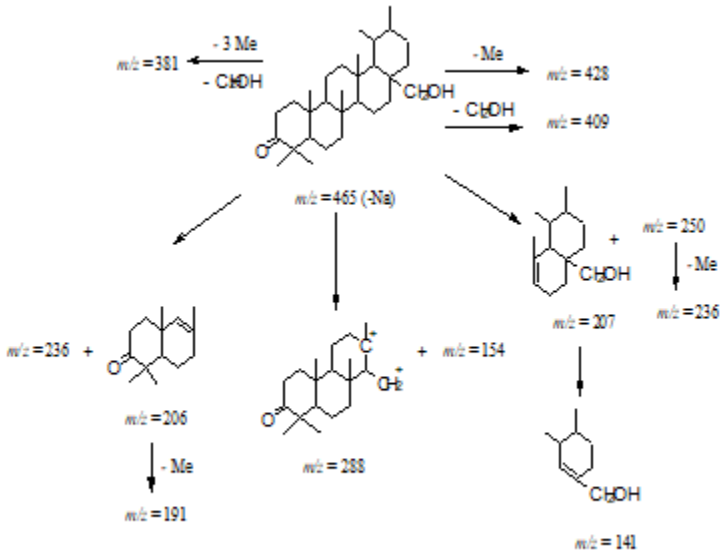

Figure 4: Structure showing the fragmentation patterns proposed

Methyl of isopropyl group was resonated at δ 0.82 as doublet at C-26 and C-27. A triplet at δ 0.85 was assigned to methyl protons of C-29. The hydroxyl protons were resonated at δ 1.58 as singlet, while a multiplet at δ 3.56 and δ 3.49 were assigned to the carbinolic protons at C-3 and C-7. A broad doublet at δ 5.35 was assigned to olefinic proton at C-4. The methylene for C-1 carbon resonated at δ 1.99 (1H, m, H-1a), and C-2 carbon resonated at δ 2.02 (2H, m, H- 2b) and δ 2.28 (1H, m, H-2a) respectively. Rest of methylene resonated at δ 1.12-1.72 (m, rest of the protons). ^{13}C NMR spectrum showed that the peaks at 11.98, 19.04, 18.78, 19.81, 19.81 and 11.86 were assigned to terminal and angular methyl carbons at C-18, C-19, C-21, C-26, C-27 and C-29 positions respectively. Peaks obtained at 140.8 and 121.6 ppm were assigned to the double bond carbons at C-4 and C-5 respectively. The carbinolic carbon at C-3 and C-7 position was resonated at 71.5 ppm and 67.77 ppm. The mass spectral analyses and combined NMR data of 4 suggested the molecular formula as $C_{30}H_{50}O_2$. IR spectrum of 4 showed typical absorption bands for hydroxyl group (3477 cm^{-1}) and for carbonyl group (1715 cm^{-1}). Bands at 2927, 2870, 1462, 1389, 1073, 981 and 482 cm^{-1} were due to –CH stretching and bending vibrations. The mass spectrum showed the molecular ion at m/z 465[-Na]. Mass fragmentation showed characteristic fragmentation of a triterpene. The important peaks were obtained at *m/z* 236 and 206. The other important fragments were obtained at *m/z* 428, 411, 290, 205 and 191. Hence EI-MS justified the nature of triterpene molecule. The ^{1}H NMR spectrum of these compound showed sharp signals for six tertiary methyls at carbon no. C-24, C-25, C-26, C-27 and C-28 appeared as singlets at δ 1.05, 1.18, 0.73, 1.00 and 0.95 respectively and doublets for C-29 and C-30 at δ 0.87 gave clear indication of compound to be ursane. The hydroxyl proton was resonated at δ 1.58 as singlet, while a broad doublet at δ 4.17 and 3.70 (1H each, d, J= 10.5 Hz, H-23), were assigned to the carbinolic proton at C-23. The methylene for C-1 carbon resonated at δ 1.96 (1H, m, H-1a), and C-2 carbon resonated at δ 2.28 (2H, m, H- 2b) and δ 2.41 (1H, m, H-2a) respectively. Rest of methylene resonated at δ 1.26-1.78 (m, rest of the protons). The chemical shifts in ^{13}C NMR spectra suggested that the molecule is triterpene and the peaks at 14.63, 17.92, 18.22, 22.25, 28.14, 18.63 and 20.23 ppm confirmed presence of seven methyls at C-24, C-25, C-26, C-27, C-28, C-29 and C-30 respectively. The carbonyl carbon C-3 was appearing at 213.04 ppm. The hydroxyl group containing carbon at C-23 position was resonated at 67.3 ppm. Thus on the basis of above evidences and comparison with literature value, 4 was characterized as 24-ol-ursen-3-one. IR spectrum of 5 showed typical absorption band at 3432 cm^{-1} suggested the presence of hydroxyl group. The absorption bands at 1731 and 1071 cm^{-1} were indicated the presence of carbonyl group and C-O linkage respectively. Bands at 1632, 1629, 1504 and 1444 cm^{-1} were indicated the presence of aromatic moiety. All these data

indicated it to be flavone molecule. The ESI-mass spectral data and elemental analyses associated to the NMR data of 5 are coherent with the molecular formula $C_{20}H_{20}O_8$. The pattern of the ESI-MS spectrum revealed that 5 was a characteristic fragmentation pattern of a flavone. The molecular ion peak appeared at *m/z* 411(-Na) and the molecular formula was found to be $C_{20}H_{20}O_8$. The prominent fragments at m/z 208 and 93. Abundant fragments at m/z 359, 326, 315, 280, 243, 231, 193, 154, 123 and 82 were indicated flavone moiety [4].

1H NMR spectrum showed showed doublets at δ 8.72 (d, J=1.58 Hz) assigned to H-3' and H-6'unsustituted system in B ring. The triplets at δ 7.59 (d, J=2.4 Hz) and 7.22 (d, J=8.14 Hz) were assigned to 4'and 5'-unsubstituted system in C-ring. Singlet's at δ 4.05 to 4.15 indicated three methoxy groups present in the molecule at C-5, C-6 and C-7 carbons. The sharp singlet at δ 2.15 was resonated for methyl protons for keto group which linked at C-8 carbon. Hydroxyl group attached to C-3 carbon resonated at δ 3.89 as doublet. Hydroxyl proton attached to C-2' carbon resonated at δ 6.35 as a singlets. The ^{13}C NMR spectrum showed that the peak at 105.0 ppm was assigned to anomeric carbon at C-10. The peak at 215 .08 ppm (C-4) indicated it as flavone carbonyl carbon. The peaks at 173.2 ppm showed presence of carbonyl group attached to C-8 carbon. The peaks at 80.35 and 79.47 ppm assigned to C-2 and C-3 respectively. Thus on the basis of above spectral evidences, 5 was characterized as Epicatechin-5, 6, 7-trimethoxy-8-methyl keton-2, 2'-diol, it is a new compound being reported for the first time by us.

IR spectrum of 6 showed typical absorption band at 3477, and 1715 cm^{-1} for the presence of hydroxyl and carbonyl group respectively. Bands at 2927, 2869, 1457, 1389, 1109, 1073, 1001 and 568 cm^{-1} were due to –CH stretching and bending vibrations. The ESI-mass spectral data and elemental analyses associated to the NMR data of 6 are coherent with the molecular formula $C_{30}H_{50}O_2$. The mass spectrum showed the molecular ion at m/z 465[-Na]. Mass fragmentation showed characteristic fragmentation of a triterpene. The important peak was obtained at *m/z* 288, 219 and 191. The other important fragments were obtained at *m/z* 428, 409, 302, 245, 236, 206, 154 and 141. The fragmentation pattern was given. The ^1H NMR spectrum showed sharp signals for six tertiary methyls at carbon no.C-23, C-24, C-25, C-26 and C-27 appeared as singlets at δ 0.95, 1.05, 1.18, 0.73 and 1.00 respectively and doublets for C-29 and C-30 at δ 0.88 gave clear indication of compound to be ursane. The hydroxyl proton was resonated at δ 1.56 as singlet, while a broad doublet at δ 3.73 (1H each, d, J= 10.5 Hz, H-28), were assigned the presence of alcohol group at C-28 carbon. The methylene for C-1 carbon resonated at δ 1.96 (1H, m, H-1a), and C-2 carbon resonated at δ 2.28 (2H, m, H- 2b) and δ

2.41 (1H, m, H-2a) respectively. Rest of methylene resonated at δ 1.22-1.78 (m, rest of the protons). The chemical shifts in ^{13}C NMR spectra suggested that the molecule is triterpene and the peaks at 28.16, 14.64, 17.93, 18.23, 22.27, 18.64 and 20.24 ppm confirmed presence of seven methyls at C-23, C-24, C-25, C-26, C-27, C-29 and C-30 respectively. The carbonyl carbon C-3 was appearing at 213.07 ppm [5]. The hydroxyl group containing carbon at C-28 position was resonated at 77.0 ppm. Thus on the basis of above spectral data and comparison with literature value 6 is an Ursen-3-one-28-ol.

CONCLUSION

On the basis of IR, 1H-NMR, 13C-NMR, mass spectral analysis and comparison by literature data, 7 was identified as β-sitosterol, 8 was identify as 3-ursenone, 9 was identified as Friedelan-3-ol and 10 was identified as Friedelan-3-one. All these 4 compounds are known compounds and found in a many plants and foods such as rice bran, wheat germ, corn oils, soybeans and peanuts and also used as anti- inflammatory effects. From the In vitro screening of compounds 1-10 against antimicrobial and antifungal showed moderate activity against *E. coli, Serratia spp.* and *S. aureus.* All of compounds showed noticeable activity against *E. coli, Serratia spp.* and *Salmonella typhimurium.*

REFERENCES

[1] David G, Liu DX, Richards RME. *Phytochemistr*y. **1996**;42(2):505-508.

[2] Jin JL, Lee YY, Heo JE, et al. *Arch. Pharm. Res.* ***2004****;* 27(4), 376-380.

[3] Aimi N, Likhitwitayawuid K, Goto J, et al. *Tetrahedron*, **1989**;45, 4125-4134.

[4] Imakura Y, Kobayashi S, Yamahara Y, et al. *Chem. Pharm. Bull.* **1985**;3396),2220-2227.

[5] Kitajima J and Tanaka Y. *Chem. Pharm. Bull.* **1993**;41(11),2007-2009.