(ii) One secondary C-methyl group (CH-CH_3), (iii) Three anomeric protons of glycosidic linkage, iv) One carbinol methine as > CHOG and v) one epoxy linkage. The above structure of Acornine 2 is also in agreement with the high resolution ^{13}C NMR spectral data described in Table 5. ^{13}C NMR data of Acornine 1 is practically completely identical with that of Acornine 2 excepting the carbon signal at C16 (data not shown) and confirms the proposed structure of Acornine 1.

Acornine 1 and Acornine 2 were subjected to electron impact mass spectral studies next. Various mass peaks obtained from these two triterpenoidal saponins and the probable interpretations of the genesis of these mass peaks are elaborated as follows: The mass spectrum [Table 4] of Acornine 1 exhibits molecular ion peak at *m/z* 928 which is in complete harmony with the molecular composition, $C_{48}H_{80}O_{17}$. The appearance of significant peaks in the mass spectrum at *m/z* 678 and at *m/z* 249 resulting from retro-Diels-Alder collapse around ring (C) under electron impact reveals the presence of oleanane type of pentacyclic triterpenoid skeleton in the compound.[21] However, the absence of any olefinic proton signal in its ^1H NMR spectrum as well as the presence of an epoxide functionality in Acornine 1 suggests the presence of an epoxide group in the compound. This assumption is further supported by the appearance of ion peaks containing ring D and E at *m/z* 249 and 219 [249-30(-CH_2-O)] in the mass spectrum. Further, the display of ion peak at *m/z* 201 (219-H_2O) by the mass spectrum indicates the presence of a hydroxyl function in the ring (D) probably at C-16 position. It may be mentioned that C-16 H proton signal probably overlapped in the region around d 3.20. Once again, ion peaks at *m/z* 782, 620, 458 and at *m/z* 457 in the mass spectrum is suggestive of the presence of gluc-gluc-rha type of glycosidic linkage. The appearance of the peak at *m/z* 678 in the mass spectrum generated by retro-Diels-Alder cleavage represents the rings A/B. The mass value of this ion peak indicates the presence of the glycosidic linkage in the rings A/B portion and its location at C3 is highly probable from biogenetic background. The mass spectrum of Acornine 2 [Table 4] is almost identical in all respects with that of its congener Acornine 1 excepting in the mass values of molecular ion peaks which appears at 16 mass units less than that of Acornine 1 is at at *m/z* 912 as well as of other ion peaks. As expected it exhibits ion peaks at *m/z* 912 (M+), 678, 233, 203, 766, 604, 442 and *m/z* 441. From the analyses of different spectral characteristics described above the most probable structures of Acornine 1 and Acornine 2 may be elaborated as shown in Figure 2. The nomenclature of the isolated compounds may be designated as follows:

Oleanane 13,28 epoxy-16 hydroxyl-3-glycoside (Acornine 1)

Oleanane 13,28 epoxy-3-glycoside (Acornine 2).

Table 4: Mass spectrum of Acornine 1 and Acornine 2

Acornine 1/Acornine 2	
Characteristic absorption band (*m/z*)	**Probable assignment**
678 and 249/678 and 233	retro-Diels-Alder collapse around ring (C) reveals the presence of oleanane type of pentacyclic triterpenoid skeleton
201 (only in Acornine 1)	[219-18 (H_2O)], presence of hydroxyl functional group in the ring (D) probably at C-16 position
219/203	[249-30 (-CH_2-O)]/[233-30 (-CH_2-O)]
782/766	[928-146 (Rha)]/[912-Rha]
620/604	[928-146+162(Rha+Gluco)]/[912-(Rha+Gluco)]
458/442	[928-146+162+162 (Rha+Gluco+Gluco)]/[912-(Rha+Gluco+Gluco)]

Rha: Rhamnose, Gluco: Glucose

Table 5: ^{13}C NMR (600 Mhz) data of Acornine 2

Triterpene portion		Sugar portion	
Carbon atom	**Chemical shift (δ)**	**Carbon atom**	**Chemical shift (δ)**
C-1	38.9	C-1	100.15
C-2	26.08	C-2	77.50
C-3	89.50	C-3	78.03
C-4	38.00	C-4	73.50
C-5	57.82	C-5	76.6
C-6	18.05	C-6	180.6
C-7	30.91	C-1'	100.30
C-8	48.73	C-2'	79.00
C-9	48.87	C-3'	76.02
C-10	36.00	C-4'	71.03
C-11	23.90	C-5'	80.00
C-12	32.65	C-6'	65.07
C-13	86.56	C-1''	100.40
C-14	44.18	C-2''	75.30
C-15	28.09	C-3''	77.10
C-16	25.08	C-4''	68.99
C-17	32.00	C-5''	69.50
C-18	49.41	C-6''	16.91
C-19	40.00		
C-20	40.03		
C-21	30.03		
C-22	36.18		
C-23	25.08		
C-24	16.50		
C-25	20.03		
C-26	18.12		
C-27	18.94		
C-28	72.17		
C-29	72.38		
C-30	24.83		

Wahidullah *et al*.,[13] isolated and characterized an oleanane triterpenoid oligoglycoside having antifungal activity from this plant which they named as corniculatonin. The deduced structure for corniculatonin[13] has similarities with that of Acornine 1 and Acornine 2. Both corniculatonin

and acornines have oleanane triterpenoid backbones. The points of major differences are however, the (i) existence of only three sugar units in cases of Acornine 1 and Acornine 2 as opposed to five in case of corniculatonin and (ii) absence of hydroxyl group (OH) in Acornine 2. In addition, Wahidullah *et al*.,[13] did not report any Gram-positive antibacterial activities for corniculatonin. Because we do not have corniculatonin we cannot determine the relative efficiency of this compound compared to the Acornines. Acornine 2 appears to be more efficient than Acornine 1 where antifungal activities are concerned. It is not clear at this time whether the absence of the –OH group in Acornine 2 is responsible for the observed differences in antifungal activities between themselves. Also, the backbone of the three compounds (corniculatonin and acornines) remaining similar to each other, yet the observation that Acornine 1 shows reduced activities compared to Corniculatonin and Acornine 2 may shed lights on the roles of side carbohydrate moieties in the observed antifungal activities and may help in the elucidation of their mechanism of action. Mention must be made here that Wahidullah *et al*.,[13] collected their plant samples from the west coast of India whereas our plant samples were collected from Sundarban estuarine areas in the east coast of India. Our findings suggest the presence in *A. corniculatum*, collected from Sundarban regions of West Bengal state of India, of two naturally occurring compounds having antifungal activities that are structurally similar to corniculatonin but not identical to it. When the bark extracts of our plant samples were run following the conditions described by Wahidullah *et al*.,[13] in a TLC experiment, we have not seen any component having the mobility characteristic even close to that of corniculatonin described by them (data not shown). Added to this, when one considers the structural similarities of corniculatonin and Acornines, it prompts us to opine that Acornine 1 and 2 may be different metabolic manifestations of corniculatonin expressed differently under different microenvironments. From that viewpoint, Acornine 1 and 2 may be regarded as novel compounds. We have named these two compounds as Acornine 1 (Oleanane 13,28 epoxy-16 hydroxyl-3-glycoside; R_f 0.36 in BAW solvent system) and Acornine 2 (Oleanane 13,28 epoxy-3-glycoside; R_f 0.32 in BAW solvent system) to distinguish them from corniculatonin described by Wahidullah *et al*.[13]. Acornine 2, in particular, was effective as antifungal against several pathogenic fungi tested and also against some Gram-positive bacteria.

CONCLUSION

We isolated two compounds having notable antifungal activities from the bark of *Aegiceras corniculatum*, a mangrove plant, found in the Sundarban estuary of India. We named the two compounds as Acornine 1 (Oleanane 13,28 epoxy-16 hydroxyl-3-glycoside) and Acornine 2 (Oleanane 13,28 epoxy-3-glycoside**).** Acornine 2 was effective as antifungal against several pathogenic fungi tested and also against some Gram-positive bacteria.

Wahidullah *et al*.,[13] collected plant samples from western coast of India whereas plant samples used in this study were collected from the Sundarban areas in the east coast of India. The differences in the microenvironments and biotic as well as abiotic stress factors in two geographically distinct coastal areas may be the reasons why the plant is synthesizing the compounds having antifungal activities in different ways. Differential gene expression under changed environments may have enabled the synthesis of slightly different compounds. Each of these compounds (corniculatonin and Acornines) may be endowed with better ability that is suited best in the particular environment in which the plant is growing for combating differential threats (biotic and abiotic) existing in two coastal areas of India having different climatic and stress conditions. Additionally, our finding strengthens the well-known belief that (i) environmental factors dictate the secondary metabolites profile of medicinal plants and (ii) the same plant species growing in different natural habitats around the world may have different metabolite profiles with respect to these compounds. Acornine 1 and Acornine 2 are previously unreported compounds having antifungal activities. Presence of both antifungal and Gram-positive antibacterial activities indicates necessity of further studies of these two compounds.

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REFERENCES

1. Tomlinson PB. The Botany of mangroves. Cambridge: Cambridge University Press; 1986.p. 414.
2. Kathiresan K, Bingham BL. Biology of mangroves and mangrove ecosystems. Adv Mar Biol 2001;40:81-251.
3. Vannucci M, editor. Mangrove management and conservation–present and future. Tokyo: U.N. University Press; 2004.
4. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of Mangrove Plants. Wetlands Ecol Manage 2002;10:421-52.

5. Gurudeeban S, Satyavani K, Ramanathan T, Balasubramanian T. Antidiabetic effect of a black mangrove species Aegiceras corniculatum in alloxan-induced diabetic rats. J Adv Pharm Technol Res 2012;3:52-6.
6. Roome T, Dar A, Ali S, Naqvi S, Choudhary MI. A study on antioxidant, free radical scavenging, antiinflammatory and hepatoprotective actions of *Aegiceras corniculatum* (stem) extracts. J Ethnopharmacol 2008;118:514-21.
7. Agoramoorthy G, Chen FA, Venkatesalu V, Kuo DH, Shea PC. Evaluation of antioxidant polyphenols from selected mangrove plants of India. Asian J Chem 2008;20:1311-22.
8. Chandrasekaran M, Venkatesalu V, Anantharaj M, Rajendran S, Prabhakar K. Studies on the antibacterial activity of a mangrove, *Aegiceras corniculatum*. J Annamalai Univ Sci 2004:169-74.
9. Vadlapudi VR, Bobbarala V. *In vitro* antimicrobial activity of two mangrove plants *Aegiceras corniculatum* and *Hibiscus tiliaceous*. Biosci Biotechnol Res Asia 2009;6:321-24.
10. Uddin SJ, Rouf R, Shilpi JA, Alamgir M, Nahar L, Sarker SD. Screening of some Bangladeshi medicinal plants for *in vitro* antibacterial activity. Orient Pharm Exp Med 2008;8:316-21.
11. Gupta VK, Roy A. Comparative study of antimicrobial activities of some mangrove plants from Sundarban estuarine regions of India. J Med Plants Res 2012;6:5480-8.
12. Giron OC, Sumera FC, Miles DH, Cajipe GJ, Chavez VB, Gomez ED, *et al*. A chemical toxicant from a mangrove plants *Aegiceras corniculatum* Blanko (Aegicerataceae). Philipp J Sci 1988;117:39-53.
13. Wahidullah S, Bhosak SH, D'Souza ML. Composition containing novel compound Corniculatonin having antifungi properties and a process for preparing the same. Council of Scientific and Research, India. US patent no. 6777004 B1; 2004.
14. Sambrook J, Russel DW. Molecular cloning, a laboratory manual. 3rd ed. New York: Cold Spring Harbor Laboratory Press; 2001. p. A2.12.
15. Bauer AW, Kirby WM, Sherries T. Antibiotic susceptibility testing by a standard single disc method. Am J Clin Pathol 1966;45:493-6.
16. Guerin-Faublee V, Muller ML, Vigneulle M, Flandrois JP. Application of a modified disc diffusion technique to antimicrobial susceptibility testing of *Vibrio anguillarum* and *Aeromonas salmonicida* clinical isolates. Vet Microbiol 1996;51:137-49.
17. Ghosh AK, Sengupta S. Studies on biochemistry of higher fungi. II. Submerged growth of a few mushrooms in synthetic media. J Food Sci Technol 1978;15:237-42.
18. Ghosh AK, Banerjee PC, Sengupta S. Purification and properties of xylan hydrolase from mushroom *Termitomyces clypeatus*. Biochim Biophys Acta 1980;612:143-52.
19. Nostro A, Germano MP, Angelo VD, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Let Appl Microbiol 2000;30:379-84.
20. Sofowara A. Medicinal plants and traditional medicine in Africa. Canada: John Wiley and Sons; 2000.
21. Budzikiewicz H, Djerassi C, Williams DH. Structure elucidation of natural products by mass spectrometry. Vol. 2, San Francisco, London, Amsterdam: Holden-Day Inc.; 1964.

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