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Spectral characterization of bioactive compounds from *Costus pictus* and *Costus speciosus*

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Abstract : The present study was made an attempt to characterize the chemical classes of the fractions showed promising biological activity against the chosen bacterial pathogens. Five chemical classes have been identified by the present study viz., 6-hydroxy-benzoxazolinone, (Z)-4-Coumaric acid 4-o-β-D-glucopyranoside, 3, 5-dimethoxy- 4-hydroxy benzoate, 2, 3-secoatisane type diterpene and 3, 4, 5-trihydroxy benzoate. Of these, 6-hydroxy-benzoxazolinone, (Z)-4-Coumaric acid 4-o-β-D-glucopyranoside and 3, 5-dimethoxy- 4-hydroxy benzoate were identified from *Costus speciosus* and 2, 3-secoatisane type diterpene and 3, 4, 5-trihydroxy benzoate were identified from *Costus pictus.* It is important to observe that, 3, 5-dimethoxy- 4-hydroxy benzoate has identified as new compound from *Costus speciosus* but the other compounds were identified previously by several authors.

Introduction

In general medicinal plants producea variety of secondary metabolites in order to treat for different types of diseases. Hence the secondary metabolite compounds are being responsible for specific physiological changes or the therapeutic action in the human body when administered as a medicament or a health supplement. The chemical constituents can serve as a marker in the finished form of herbal drug. One of the best methods of standardizing herbs or herbal formulations based on modern scientific herbal characteristics is chromatography. The insulin plant namely *Costus pictus* and *Costus speciosus* are commonly known as fiery *Costus* or Spiral flag. It is widely grown in South India as an ornamental plant in gardens and also in many places. This plant is mainly used to control diabetes mellitus, by taking one leaf daily to maintain their blood sugar level by diabetic people. Leaves were known to be effectively used for treating diabetes by the tribal people of Kolli hills of Namakkal district, Tamilnadu. Themethanolic extract was found to havemore number of phytochemicals such as carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, and flavonoids in another study (1)(4). Preliminary phytochemical evaluation of Insulin plant revealed that the leaves contain 21.2% fibers. *Costus* are used to treat for fever and rhizomes extract is used for treating boils. It is also used to make sexual hormones and contraceptives (26)(17). Leaves are used for scabies and stomach ailments. Leaves are ground into paste and applied to the fore head to bring down high fever. Besides rhizomes, stems are also used for treating blisters and burns. Roots are used against snake bite (6)(18).

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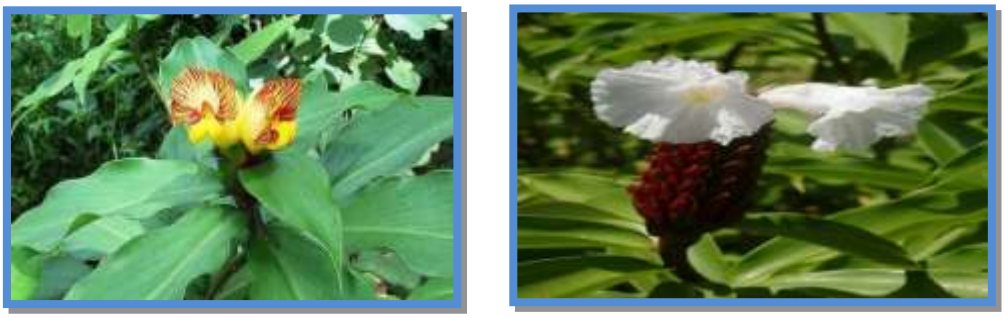
The present study was also made an attempt to characterize the chemical classes of the fractions showed promising antibacterial activity against the chosen bacterial pathogens.

Materials and Methods

Collection and extraction of bioactive compounds

Fresh matured leaves of *Costus pictus* and *Costus speciosus* were collected from Western Ghats,Tamilnadu (Fig 1). The collected leaves were washed 3 times in sterile distilled water to remove the unwanted particles. After washing, leaf samples were shade dried and powdered. The powdered leaves 500g was taken and soaked in 1L of ethanol water mixture (3:1) for 10 days in an air tight clean glass container with continuous shaking everyday to enhance the maximum extraction of bioactive compounds (3)(4). Later the extracts were filtered through muslin cloth and kept in the rotary flash evaporator (with solvent trap) to obtain solvent free extract residue and stored in refrigerator for further use. The presence of saponins, tannins, alkaloids, flavonoids, anthraquinones, glycosides and reducing sugars in the extract were tested by following simple and standard qualitative methods. Triplicates were maintained throughout the experiment to get concordant values and the results were statistically analyzed for significance.



Costus pictus **Costus speciosus**

Figure 1: Costu spictus & Costus speciosus

Fractionation by Silica column chromatography

The obtained colloidal form of extract were suspended in water separately and defatted with diethyl ether (10). The aqueous layer of extracts were subjected to column chromatography packed with 500g of silica gel (230-400 mesh)(MERCK) with the maximum height of 50cmand eluted successively with 30ml of n-hexane, benzene, chloroform, acetone, ethanol and water. MERCKAR grade solvents were used throughout the study.

The corresponding fractions of 1-5 (63g, n-hexane), 6-10 (81g, benzene), 11-15 (10.6g, chloroform), 16-20 (14.5g, acetone), 21-25 (106g, ethanol), 26-30 (145g, water) were collected from *Costus speciosus* and 31-35 (52g, n-hexane), 36-40 (31g, benzene), 41-45 (16g, chloroform), 46-50 (66g, acetone), 51-55 (122g, ethanol), 56-60 (155g, water) were collected from *Costus pictus.* The obtained fractions were labelled and stored at -80^{0}C (SANYO-JAPAN) for further use.

UV Spectral Analysis

Ultra violet spectrum (λ_{max} in nm) was recorded on a UV/VIS spectrophotometer(CyberUV-1) for the active column chromatographic fractions of *Costus speciosus* and *Costus pictus* to find out the conjugation if any present in the bioactive compounds.

^{1}H-Nuclear magnetic resonance Spectrum Analysis

The ^{1}H- Nuclear magnetic resonance (NMR) Spectra of the active column chromatographic fractions of *Costus speciosus* and *Costus pictus* was obtained in a Bruker spectrometer 400 MHz. The samples was dissolved in dimethyl sulfoxide (DMSO)to provide a field-frequency lock signal and contained 1% tetramethylsilane (TMS) to provide an internal chemical shift standard(Aldrich). One dimensional ^{1}H-NMR

spectra was acquired using a narrow bore probe. Typical experimental conditions included two repetitions between successive acquisition 32768 time domain points and spectral width of 8223.685 Hz. Data processing and ^{1}H peak integration were done using XWINNMR software (Bruker, Karlsrube, Germany) running in an R-4000 workstation (Silicon Graphics, Mountain view, CA, USA). This methodology is used to identify the position, type and neighboring number of protons in the bioactive compounds.

^{13}C- Nuclear magnetic resonance Spectrum Analysis

The ^{13}C- Nuclear magnetic resonance (NMR) Spectra of the active column chromatographic fractions of *Costus speciosus* and *Costus pictus* was obtained in a Bruker spectrometer 100 MHz. The samples was dissolved in dimethyl sulfoxide (DMSO)to provide a field-frequency lock signal and contained 1% tetramethylsilane (TMS) to provide an internal chemical shift standard(Aldrich). One dimensional ^{13}C-NMR spectra was acquired using a narrowbore probe. Typical experimental conditions included two repetitions between successive acquisition 65536 time domain points and spectral width of 24038.461 Hz. Data processing and ^{13}C peak integration were done using XWINNMR software (Bruker, Karlsrube, Germany) running in an R-4000 workstation (Silicon Graphics, Mountain view, CA, USA). This methodology is used to know the carbon skeleton of bioactive compounds.

FT-IR Spectrum Analysis

FT- IR spectral (υ_{max} in cm^{-1}) analysis was carried out for the active column chromatographic fractions of *Costus speciosus* and *Costus pictus* to detect the functional group of the bioactive compounds. It was recorded in KBr on a JASCO FT/IR-460 plus spectrophotometer.

Results and Discussion

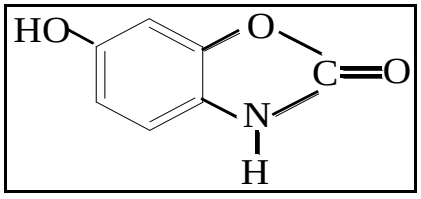
Identification of compounds

Identification of compounds in *Costus speciosus*

The structure of compounds 1, 2 and 3 were determined from fractions 15, 25 and 30 from eluted *Costus speciosus* by using UV,IR,^{1}H- and ^{13}C-NMR and the compounds 4 and 5 from fractions 41 and 60 from *Costus pictus* by using UV,IR, ^{1}H- and ^{13}C-NMR.The compounds were identified as 6-hydroxy-benzoxazolinone, (Z)-4-Coumaric acid 4-o-β-D-glucopyranoside and 3, 5-dimethoxy- 4-hydroxy benzoate and their molecular structures were elucidated from *Costus speciosus*.

Identification of compound - 1

Compound 1 was identified as 6-hydroxy-benzoxazolinone and the results are given below. Compound **1** is a crystalline solid, UV (λ max) 274 nm (Fig. 2). InC-NMR spectrum (Fig. 3) seven carbons were resolved as three aromatic methines (δ 99.0, 111.0 and 111.5) assigned to C-7, C-5 and C-4 respectively, three quaternary aromatic carbons (δ 130.0, 146.2 and 154.9) attributed to C-9, C-8 and C-6 respectively and a fourth quaternary carbon at δ 157.8 assigned to the lactone carbonyl. The ^{1}H-NMR spectrum (Fig. 4) showed at δ 6.65, 6.80 and 6.58 indicates a trisubstituted aromatic system. The IR spectrum (υ_{max} in cm^{-1}) in KBr (Fig. 5) showed peaks of variable intensities at 3435, 1770, 1737, 1625 and 1462 indicating hydroxyl, amide carbonyl and aromatic ring group respectively. From these results, the structure of compound 1 is identified as the 6-hydroxy-benzoxazolinone.



Sample No: 15

Physical and spectral data of Compound 1 were found to be identical with those reported from *Acanthus arboreus* (13). Further reported the same compound as a novel product from *Echinops echinatus* under the name *echinacin* (24).Our assignments were in the spectra, the compound 1 was identified and predicted as the 6- hydroxy-benzoxazolinone.

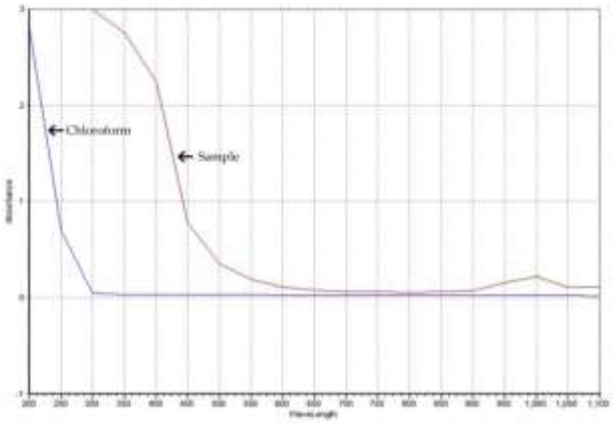


Fig.2: UV analysis of 6-hydroxy-Benzoxazolinone (Compound 1)

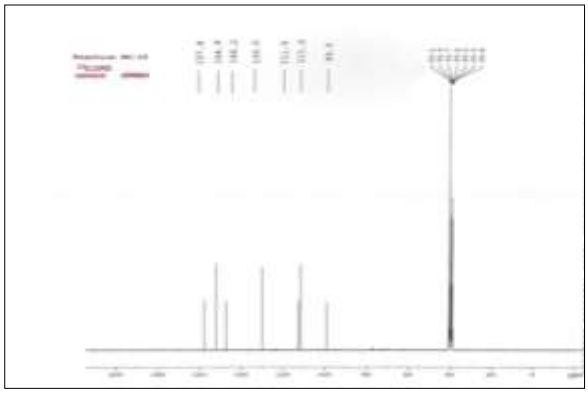


Fig.3: ^{13}C-NMR analysis of 6-hydroxy-Benzoxazolinone (Compound 1)

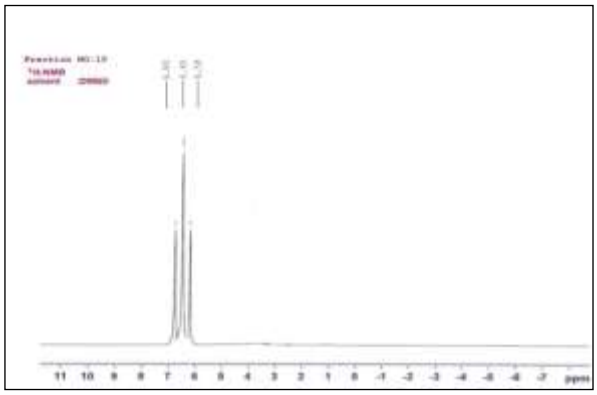


Fig. 4:^{1}H-NMR analysis of 6-hydroxy-Benzoxazolinone (Compound 1)

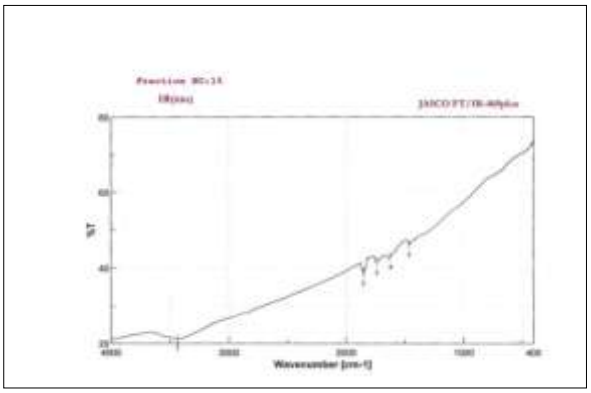
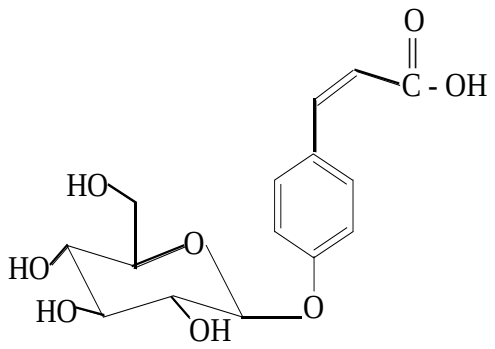


Fig.5: FT-IR analysis of 6-hydroxy-Benzoxazolinone (Compound 1)

Identification of compound - 2

Compound 2 was identified as (Z)-4-Coumaric acid 4-o-β-D-glucopyranoside and the results are given below.Compound **2** is an amorphous powder, UV (λ max) 372 nm (Fig. 6). The H- and C-NMR spectral data of 2 exhibited a nine-carbon aromatic aglycone and a monosaccharide unit δ H 5.20 and δ C 100.4 (Fig. 7). ^{1}H-NMR spectral data of 2 revealed at δ 7.14 and 7.50 (Fig. 8) indicating the presence of a 1, 4-disubsituted symmetrical aromatic ring in the aglycone moiety, two coupling olefinic protons at δ 6.08 and 6.50 suggested a Z-form double bond. Moreover, the quaternary carbon at δ 177.8 suggested the presence of a carboxyl group. The IR spectrum (υ_{max} in cm^{-1}) in KBr (Fig. 9) showed peaks of variable intensities at 3377, 2927, 1698, 1603, 1516, 1446, 1366 1275, 1159, 1063, 1039, 912 and 813 indicating hydroxyl, carboxyl, carbonyl, aromatic ring, olefinic and C-O-C group respectively. From these results, the structure of the aglycone is identified as (Z)-4-coumaric acid. Therefore, the structure of compound 2 is assigned as **(Z)-4-Coumaric acid 4-o-β-D-glucopyranoside**. 4-Coumaric acid is an important biosynthetic precursor of many natural products, which include coumarin, lignan, flavonoid and phenylethanoid.



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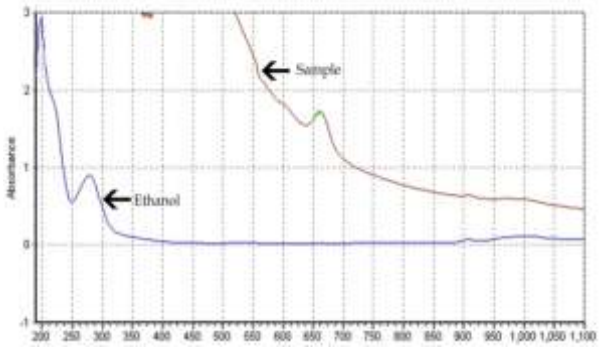


Fig.6:UV analysis of (Z)-4-Coumaric acid 4-O-β-D-glucopyranoside (Compound 2)acid

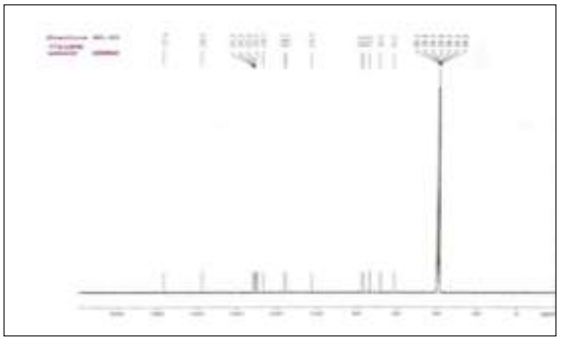


Fig.7: ^{13}C- NMR data of (Z)-4-Coumaric acid 4-O-β-D- glucopyranoside (Compound 2)

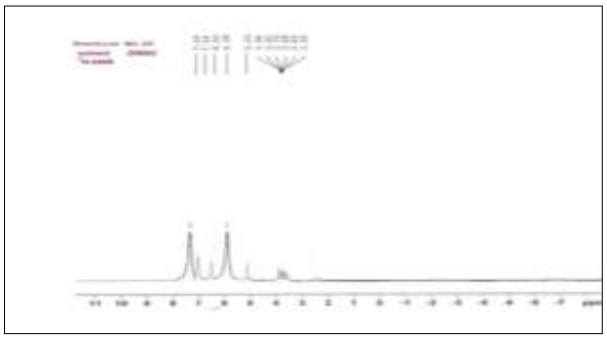


Fig.8: ^{1}H-NMRdata of (Z)-4-Coumaric acid 4-O-β-D-glucopyranoside (Compound 2)

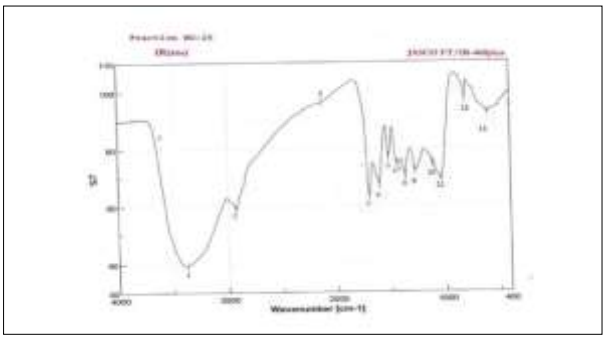
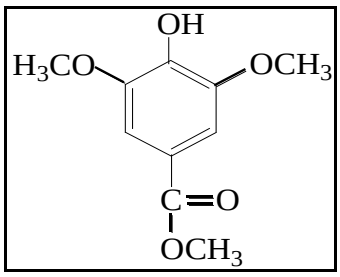


Fig.9: FT-IR data of (Z)-4-Coumaric acid 4-O-β-D-glucopyranoside (Compound 2)

Identification of compound - 3

Compound 3 was identified as 3, 5-dimethoxy- 4-hydroxy benzoate and the results are given below. Compound **3** is an amorphous powder, UV (λ max) 368 nm (Fig.10). The ^{13}C- NMR spectrum (Fig. 11) revealed the presence of 1, 3, 4, 5-tetrasubstituted symmetrical aromatic rings, two methylenes (δ 61.2 and δ 70.2), four methines (δ 51.8, δ 54.9, δ 83.3 and δ 83.4), which have two sets of equivalent methoxy carbons (δ 56.1) and one ester carbonyl carbon (δ 166.4). Its ^{1}H-NMR spectrum (Fig. 12) showed the presence of ester methyl signals at δ 2.60, aromatic hydroxyl group at δ 7.76 and two methoxy groups at δ 3.68 and δ 3.70. Its IR spectrum (υ_{max} in cm^{-1}) in KBr (Fig. 13) showed peaks of variable intensities at 3392, 1707, 1617, 1443 and 1214 indicating hydroxyl, ester carbonyl, aromatic ring and C-O-C group respectively. From these results, the structure of compound 3 is identified as **3, 5-dimethoxy- 4-hydroxy benzoate**.



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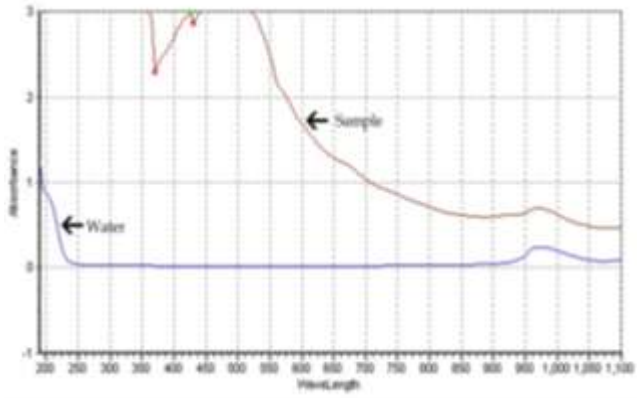


Fig.10: UV analysis of 3, 5-dimethoxy-4-hydroxy benzoate (Compound 3)

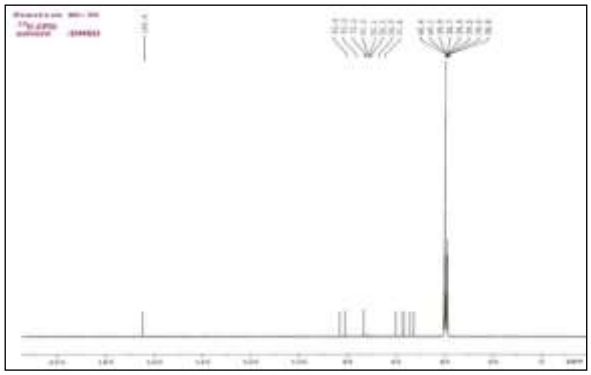


Fig.11: ^{13}C-NMR data of 3, 5-dimethoxy-4-hydroxybenzoate (Compound 3)

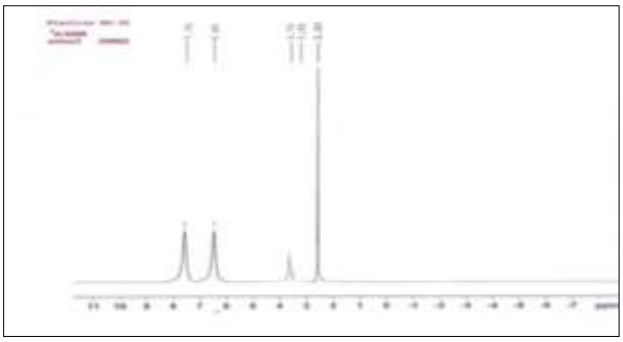


Fig.12: ^{1}H-NMR data of 3, 5-dimethoxy-4-hydroxy benzoate (Compound 3)

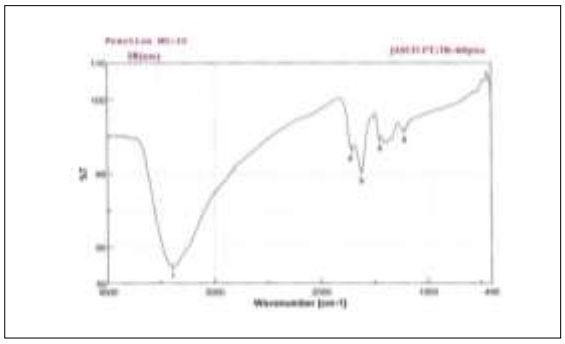


Fig.13: FT-IR data of 3, 5-dimethoxy-4-hydroxy benzoate (Compound 3)

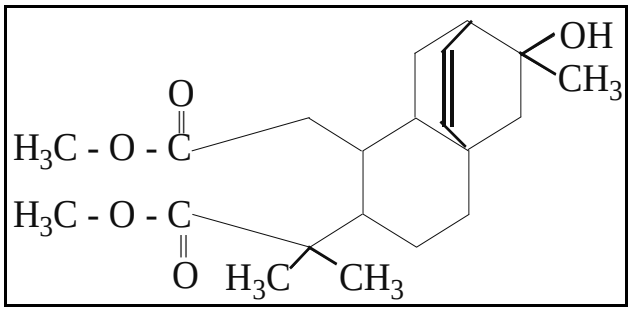
Identification of compound

The structure of compounds 4 and 5 from fractions 41 and 60 from Costus pictus by using UV-IR, ^{1}H- and ^{13}C- . The compounds were identified as 2, 3-secoatisane type diterpene, 3, 4, 5-trihydroxy benzoate and their molecular structures were elucidated from Costus pictus.

Identification of compound - 4

Compound 4 was identified as 2, 3-secoatisane type diterpene. Compound 4, is white needle crystal, UV (λ max) 272 nm (Fig.14). The ^{13}C- NMR spectrum (Fig.15) revealed the presence of two carbonyl groups (δ 172.1 C-2; 179.2 C-3), two methoxy carbons (δ 50.9, 51.8) three methyl carbons at δ 46.0, 46.i and 73.9,one pair of tri substituted double bond carbon at δ 131.8 and 18.6 and three quaternary carbons at δ 41.1, 42.0 and 46.1. The ^{1}H- NMR spectrum (Fig.16) displayed six methyl as singlet, of which two were parts of the ester methyls (δ 3.52, 3.64) and four were identified as tertiary groups (δ 1.12, 1.21, 1.24 and 1.54). The olefinic

proton at δ 5.83 was observed as singlet which indicated the presence of a trisubstituted olefin. Its IR spectrum (υ_{max} in cm^{-1}) in KBr (Fig.17) showed peaks of variable intensities at 3389, 2925, 2847, 2126, 1694, 1627, 1492, 1402, 1338, 1278, 1161, 1042, 933, 899, 811, 718, 632, 566, 431 and 413 indicating hydroxyl, carbonyl, ester and olefinic group respectively . From these results, the structure of compound 4 is identified as 2, 3-secoatisane type diterpene.



Sample No: 41

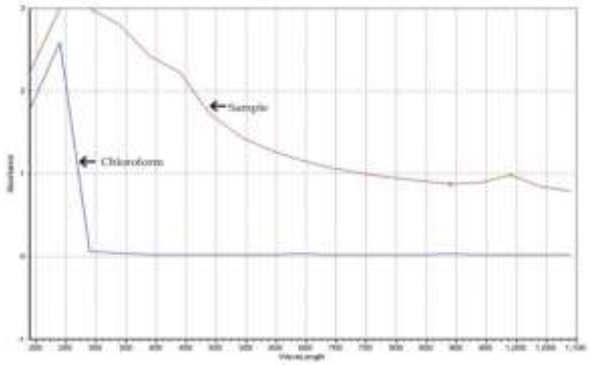


Fig.14:UV analysis of excoecarin N (Compound 4)

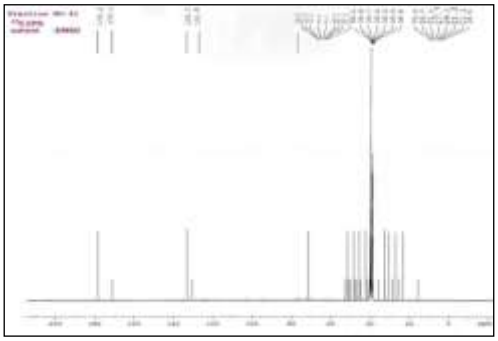


Fig. 15: ^{13}C- NMR data of excoecarin N (Compound 4)

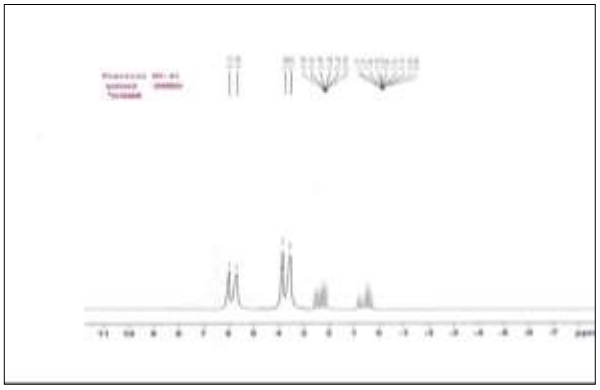


Fig.16: ^{1}H- NMR data of excoecarin N (Compound 4)

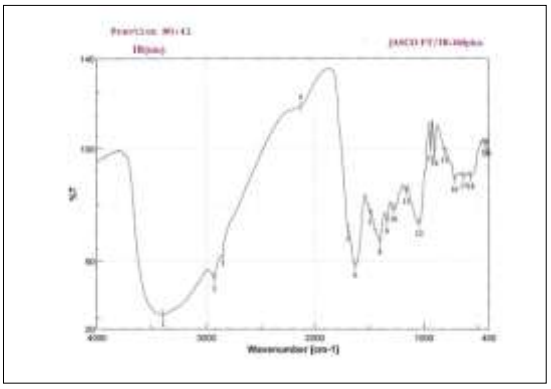
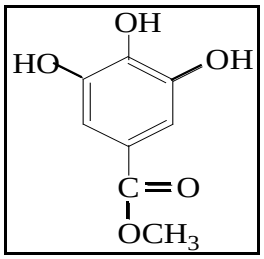


Fig.17: FTIR data of excoecarin N (Compound 4)

Identification of compound - 5

Compound 5 was identified as 3, 4, 5-trihydroxy benzoate and the results are given below.Compound 5 is an amorphous powder, UV (λ max) 365 nm (Fig.18). The ^{13}C- NMR spectrum (Fig.19) revealed the presence of 1, 3, 4, 5-tetrasubstituted symmetrical aromatic rings, two methylenes (δ 61.2 and δ 70.2), four methines (δ 51.8, δ 54.9, δ 83.3 and δ 83.4), methoxy carbons (δ 56.1) and one ester carbonyl carbon (δ 166.4). The ^{1}H-NMR spectrum (Fig.20) showed the presence of ester methyl signals at δ 2.60, hydroxyl group at 4.41, 4.42, 4.92 and aromatic group at δ 7.76. The IR spectrum (υ max in cm-1) in KBr (Fig.21) showed peaks of

variable intensities at 3390, 1694, 1628, 1338and 1279 indicating hydroxyl, carbonyl, methoxy and aromatic ring group respectively. From these results, the structure of compound 5 is identified as the 3, 4, 5-trihydroxy benzoate.



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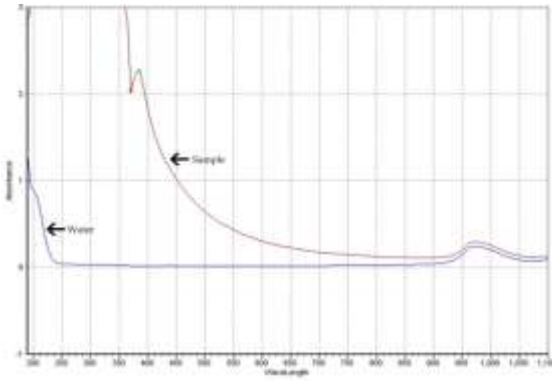

Fig.18: UV analysis of 3, 4, 5-trihydroxy benzoate(Compound 5)

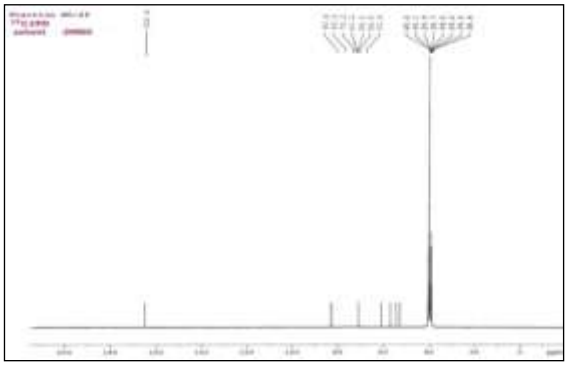

Fig.19: ^{13}C-NMR data of 3, 4, 5-trihydroxy benzoate (Compound 5)

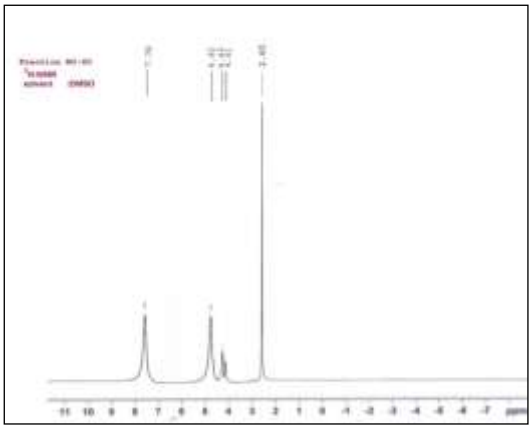

Fig.20: ^{1}H- NMR data data of 3, 4, 5-trihydroxy benzoate (Compound 5)

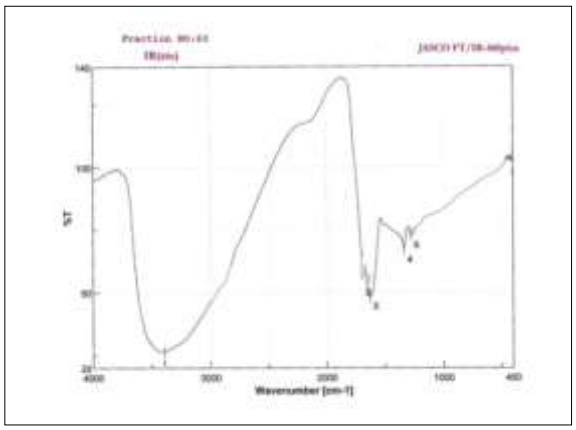

Fig.21: FT-IR data data of 3, 4, 5-trihydroxy benzoate (Compound 5)

Discussion

The present study also made an attempt to findout the anti-inflammatory and anti-analgesic property of chosen sugar plants for the possible identification of natural medicines for the treatment of arthritis. Denaturation of proteins is a well documented cause of inflammation (21). Leaf extract of *C. pictus* and *C. speciosus* at different concentrations provided significant protection against denaturation of proteins. Production of auto antigens in certain inflammatory diseases may be due to in vivo denaturation of proteins. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding (5)(11)(2). From the results of present study it can be stated that ethanolic extract of *C. pictus* and *C. speciosus* leaves are capable of controlling the production of auto antigen and inhibits denaturation of protein in

inflammatory disease.It has been reported that the viscosities of protein solutions increase on denaturation (Anson and Mirsky, 1932)(19). This decrease in viscosities may be due to decrease in concentration of test extract/drug in reaction mixture, which resulted in decreased viscosity; and/or other uncertain physico-chemical factors. Similar to studies by (20),(14);(12) suggested that the high membrane stabilizing activity of the extract of *Celosia argentea* which has potential to protect the erythrocyte membrane from free radical damage (16)(9)(10). The membrane stabilizing activity of the extract may be due to the presence of flavonoids, alkaloids, tannins and or saponins present in *C. pictus* and *C. speciosus* leaves.

The bioactivity of plant products mainly depends on the amount of the major active constituents. The data of our studies suggests that *C. pictus* and *C. speciosus* leaves extracts showed significant anti-inflammatory activity. The bioactivity of plant products mainly depends on the amount of the major active constituents. Based on the above findings, it can be concluded that the anti-diabetic activity of *Costus* species could be due to the presence of a phytochemical flavonoids in the plant. However, the above mentioned active constituent has to be isolated, characterized and evaluated for anti-diabetic activity in comparison with reference compound. To the best of our knowledge, this is the report on anti-diabetic and phytochemical investigation of *C. pictus* and *C.speciosus* even though little peripheral information and lacking scientific reported study on these plant properties based on anti-diabetic activity despite its wide usage as medicinal plant.

The rhizomes of the genus *Costus* are the major source of a compound, diosgenin(22)(4) reported diosgenin as the major constituent isolated from rhizomes of Costus species. Diosgenin was also reported from other parts of *Costus* such as leaves, stems and flowers (23). Other constituents isolated from *Costus species* are Tigogenin, dioscin, gracillin β-sitosterolglucoside (7)(16)(9). The different species of *Costus* vary in flowers, leaves and bracts. In some varieties, flowers and bracts look like cones, while others are shaped like pineapple or soft crepe coming out of green cones. Some leaves have furry or velvety textures on the back, while others are smooth and purplish (Anonymous, 1988). It is also used to make sexual hormones and contraceptives (26)(17). Leaves are used for scabies and stomach ailments. Besides rhizomes, stems are also used for treating blisters and burns. Roots are used against snake bite (6)(18).*Costus* is traditionally used as a medicinal herb mainly for its tonic, stimulant, carminative, diuretic, digestive and antiseptic properties. The rhizome is used internally in the treatment of abdominal pain, chest pains, liver problems, jaundice, gall bladder pain etc (25).

In the present study compound 1, 2,3 were eluted from fractions 15,25,30 from the extract of *Costus specious* and fractions 41 and 60 from *Costus pictus*. From the compound 1, 6-hydroxy-benzoxazolinone was identified. The same compound was previously reported in Acanthus arboreus(13) and *Echninops echinatus* (24). From Compound 2, (Z)-4-Coumaric acid 4-o-β-D-glucopyranoside, compound 3 was identified as 3, 5-dimethoxy- 4-hydroxy benzoate,compound 4 was identified as 2, 3-secoatisane type diterpene and compound 5 wasidentified as the 3, 4, 5-trihydroxy benzoate. These compounds act as precursor of many natural products includes coumarin, flavonoid, lignan and phenylethanoid. It is important to observe that, 3, 5-dimethoxy-4-hydroxy benzoate has identified as new compound from *Costus speciosus* but the other compounds were identified previously by several authors.

Conclusion

From the present study it has been concluded that, the identified compounds from *Costus speciosus* and *Costus pictus* has unique chemical compounds which could be used effectively for the treatment of variety of human and veterinary diseases. Further investigation is in progress to findout the effectiveness of the compounds for other diseases too.

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