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NEW REPORT ON EICOS-5-ENOIC ACID IN *BLECHNUM ORIENTALE* L.

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ABSTRACT

Present day hope that many people join in studying the various phytochemicals and their bioactivities of ferns and fern allies for human life. As a result, more people could enjoy a healthy and eco-friendly life with ferns and fern allies. Hence, in the present investigation, an attempt has been made to study the phytoconstituents of *B. orientale*. The compound such as Eicos-5-enoic acid was isolated by spectral analysis i.e., IR, MASS, C^{13} NMR and ^{1}H NMR for structural elucidation. So, certain functional activities for human life and possibilities of industrial application of *B. orientale* were analyzed for scientific exploration with isolated phytochemicals.

KEYWORD: Pteridophytes, *Blechnum orientale*, Phytoconstituents.

INTRODUCTION

Most people think that there are limited uses for ferns. However, these plants have given many health benefits to humans since ancient times. Not surprisingly, herbal medicines of Chinese, Indian, and Native American peoples include ferns. These cultures have used them for food, tea, and drugs. Compared to flowering plants, ferns and fern allies have limited use to human health in modern times. Plants normally produce various secondary metabolites not only to adapt to their environment but also to defend themselves against biotic or abiotic stress, such as high light intensity, extremely high or low temperature, high salinity, drought and natural enemies. To provide protection against adverse effects of their environment, plants have the tendency to produce many kinds of secondary metabolites in severe conditions (Bennett and Wallsgrove, 2006). These metabolites are polyphenols, flavonoids, terpenoids, steroids, quinones, alkaloids, polysaccharides and so on (Swain, 1977). These metabolites are also engaged with the color, flavor and aroma of plants. These functional metabolites have properties, which prevent and cure various diseases as well as aging in mammals including humans.

Phytochemistry is one of the more fashionable and rapidly expanding areas of plant taxonomy which utilizes chemical information to improve the classification of plants. The origin of chemotaxonomy may date back to thousands of years i.e. from the time of using wild plants as a source of medicine. Many ferns, among many other plants, were used for medicinal purposes by the early Greeks and Romans and through the middle ages. Dioscorides, a first century botanist, noted the use of spleenworts for curing meladies of the spleen, and the generic name *Asplenium* alludes to this early practice (Irudayaraj and Patric-Raja, 1998). The phytochemistry of South Indian ferns was also explored by Manickam and his team including Joseph *et al* (1991, 1993), Jesudass *et al.* (1992, 2001) and Raja *et al.* (1995). *Blechnum orientale* L. leaves are traditionally used as poultice to treat boils, blisters or abscesses and sores as a diaphoretic, and to treat stomach pain, skin infections, ring worms, diarrhea, urinary bladder complaints, sterilization of women and to stop wound bleeding, In addition they possess anthelmintic, anti-fungal and anti-bacterial (Deepa *et al.*, 2013), antiviral and anti-cancer, antioxidant activities (Deepa *et al.*, 2013), cytotoxicity properties. The leaf is also boiled and eaten as vegetable by the natives (Maridass and Ghantikumar, 2008; Sharief and Rao, 2007; Lai *et al.*, 2010).

A number of publications are available on the phytochemical and antimicrobial activity of pteridophytes at the global level but there are only limited publications on the *B. orientale.* Hence in the present investigation, an attempt has been made to study the phytoconstituents of *B. orientale*.

MATERIALS AND METHODS

Dried powder of leaf was extracted successively with pet ether, chloroform and methanol using soxhlet extraction unit for 18 h as per standard procedure. The extract was dried under reduced pressure to evaporate the solvent

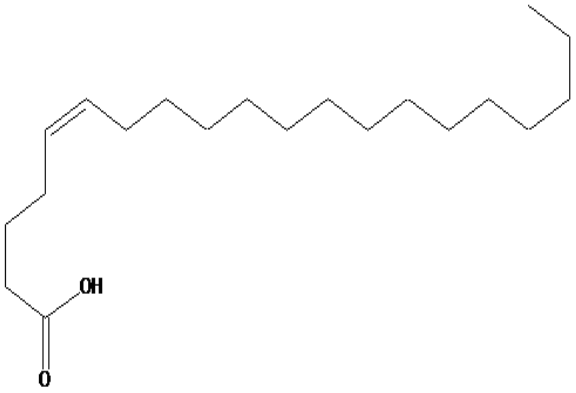
and dried mass (15 gm) was taken further for isolation work. The methanolic extract of *B. orientale* was subjected to adsorption chromatography, adsorbed sample kept for complete drying and later used for column elution.

Column was packed with slurry of silica gel of mesh size 60-120 (Sd fine chemicals, Mumbai) with chloroform. Column length was 100 cm and diameter is 3 cm. on top of silica bed, activated sample was loaded and cotton was placed on top of it to avoid any disturbance to the sample bed. Initially Chloroform solvent was eluted for small quantity for correct distribution of activated sample in the column and later with two solvent combinations with increasing order of polarity was used. Column elution was started with chloroform and methanol combinations fractions were collected in 15 ml portions.

During the above column elution process, from the fraction chloroform: methanol: 4:6 fractions were shown similar banding pattern with less bands so it was combined and kept for evaporation to dryness in room temperature. Later the residue was precipitated with acetone. The precipitated part was separated and clear solvent was kept for evaporation and residue was sent for spectral analysis. The remaining fractions were not worked out because of lower yield as well impurity. The compound was isolated by spectral analysis i.e., IR, MASS, C^{13} NMR and ^{1}H NMR for structural elucidation (Hazra *et al*., 2007; Cai *et al*., 2002; He, 2000; Tsao and Deng, 2004).

RESULTS AND DISCUSSION

The mass spectrum has shown a m/z value of 310 suggesting a molecular formula of $C_{20}H_{38}O_2$. The ^{1}H-NMR spectrum shows a triplet at δ 0.99 for three protons due to a terminal methyl group adjacent to a methylene group. The multiplet at δ 5.80 shows the presence of unsaturated protons. The triplet signal at δ 2.06 for two protons shows the presence of a methylene group adjacent to a carbonyl group. A pair of strong singlets at δ 1.25 and 1.31 and a multiplet at δ 1.73 is due to the presence of long chain methylene groups in the compound. ^{13}C-NMR spectrum exhibits a signal at δ 181 for an acid carbonyl group. The pair of signals at δ 129.72 and 130.08 confirms the presence of the unsaturated carbon atoms. The signal at δ 12.08 for a methyl group and the couple of signals between δ 21.68 to 31.04 due to long chain methylene groups confirms the assignment made in the ^{1}H-NMR spectrum. Based on the above data the structure of the compound may be proposed as Eicos-5-enoic acid (Fig. 1 to 4).



Eicos-5-enoic acid

Eicos-5-enoic acid or Eicosapentaenoic acid (EPA or also icosapentaenoic acid) is an omega-3 fatty acid. It also has the trivial name timnodonic acid. In chemical structure, EPA is a carboxylic acid with a 20-carbon chain and five *cis* double bonds; the first double bond is located at the third carbon from the omega end. EPA is a polyunsaturated fatty acid (PUFA) that acts as a precursor for prostaglandin-3 (which inhibits platelet aggregation), thromboxane-3, and leukotriene-5 groups (all eicosanoids).

It is obtained in the human diet by eating oily fish or fish oil, e.g. cod liver, herring, mackerel, salmon and sardine and various types of edible seaweed. It is also found in human breast milk. However, fish do not naturally produce EPA, but obtain it from the algae they consume (Yvonne, 2008). It is available to humans from some non-animal sources commercially, from microalgae. Microalgae are being developed as a commercial source (Jess, 2007). EPA is not usually found in higher plants, but it has been reported in trace amounts in purslane (Simopoulos and Artemis, 2002).

The US National Institute of Health's Medline Plus lists medical conditions for which EPA (alone or in concert with other ω-3 sources) is known or thought to be an effective treatment (Annon., 2006). Most of these involve its ability to lower inflammation. Among omega-3 fatty acids, it is thought that EPA in particular may possess some beneficial potential in mental conditions, such as schizophrenia (Peet *et al*., 2001; Song and Zhao, 2007). Several studies report an additional reduction in scores on symptom scales used to assess the severity of symptoms, when additional EPA is taken.

Studies have suggested that EPA may be efficacious in treating depression (Huan *et al.*, 2004). A study found that patients taking omega-3 supplements with a higher EPA:DHA ratio experienced fewer depressive symptoms (Martins, 2009). One study also reports the remission of depressive symptoms and decrease in lateral ventricular volume of a patient who suffers from chronic fatigue syndrome after taking high-EPA essential fatty acid supplement (Puri *et al.*, 2004).

EPA has an inhibitory effect on CYP2C9 and CYP2C19 hepatic enzymes. At high dose, it may also inhibit the activity of CYP2D6 and CYP3A4, important enzymes involved in drug metabolism (Yao *et al.*, 2006). Earlier reports suggest that EPA improves the response of patients to chemotherapy, possibly by modulating the production of eicosanoid (Hardman, 2004). El-Mowafy *et al.* (2011) describes, for the first time, a prominent protection by EPA against valproate (VPA)-induced hepatic dysfunction, necrosis, and steatosis. Given that VPA is commonly used in mood disorders, this may offer protection against intentional or unintentional overdose. Further, this same study showed a synergistic effect on raising seizure threshold (in pentylenetetrazol mouse convulsion model) when EPA and VPA are used concomitantly. So, certain functional activities for human life and possibilities of industrial application of *B. orientale* were analyzed for scientific exploration with these isolated phytochemicals.

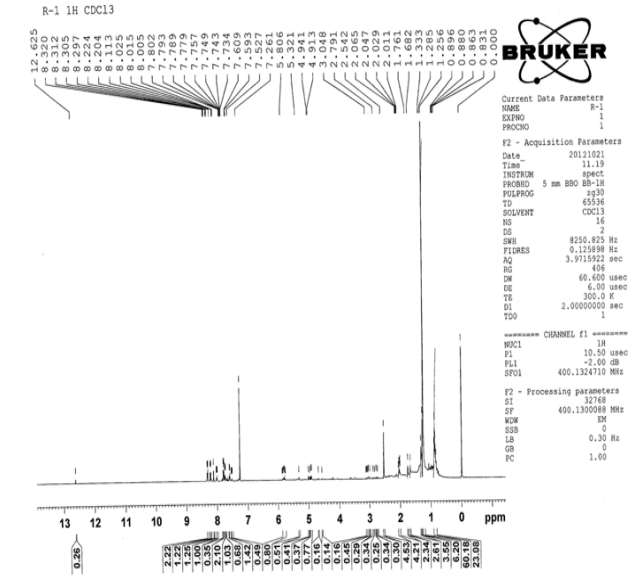


Fig. 1. ^{1}H spectrum of Eicos-5-enoic acid

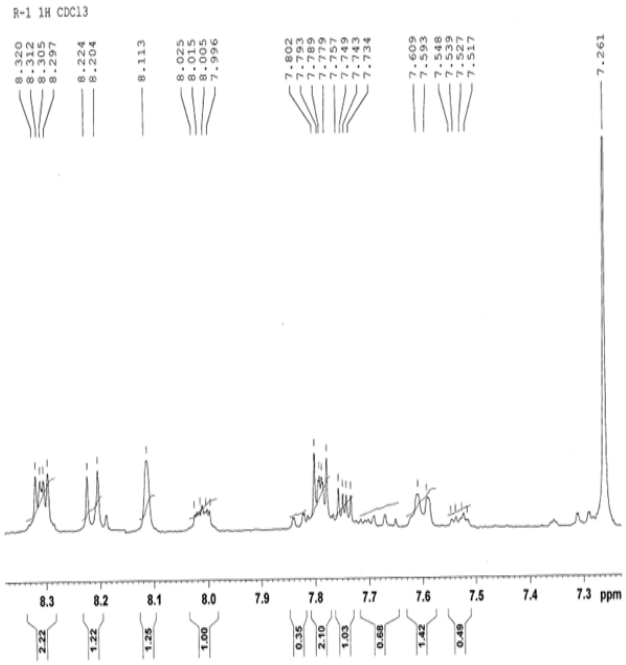


Fig. 2 ^{13}C-NMR spectrum of Eicos-5-enoic acid

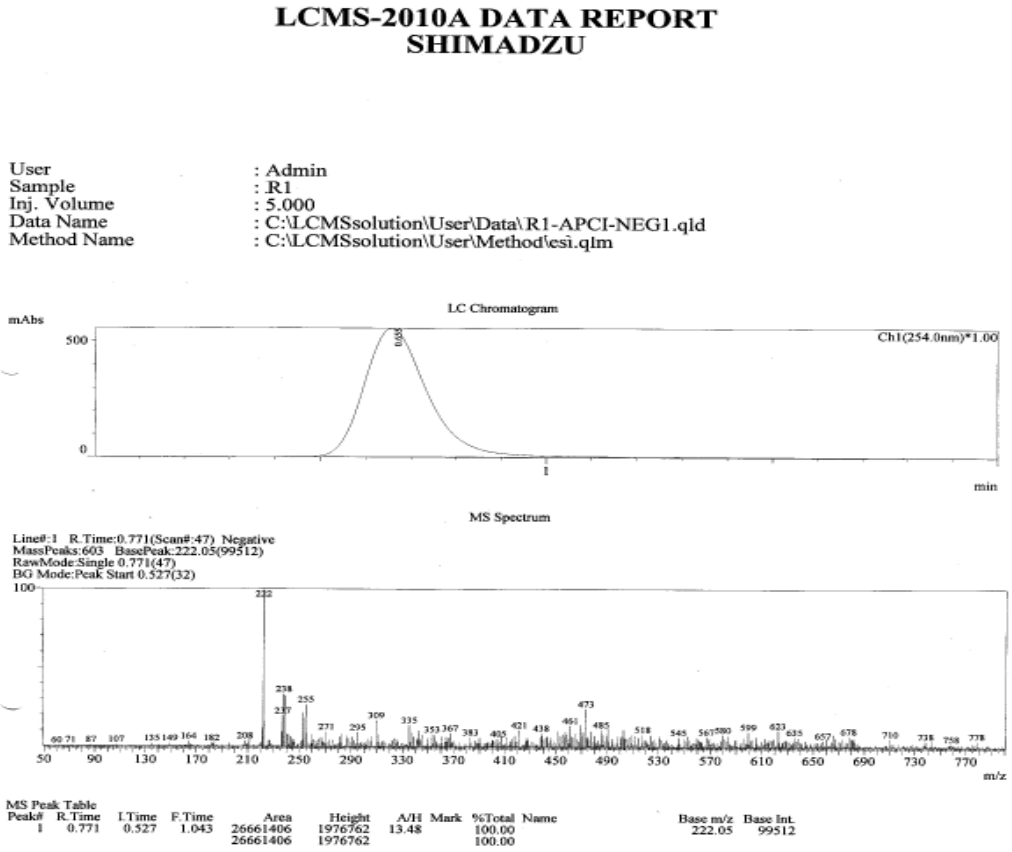


Fig: 3. LCMS of Eicos-5-enoic acid

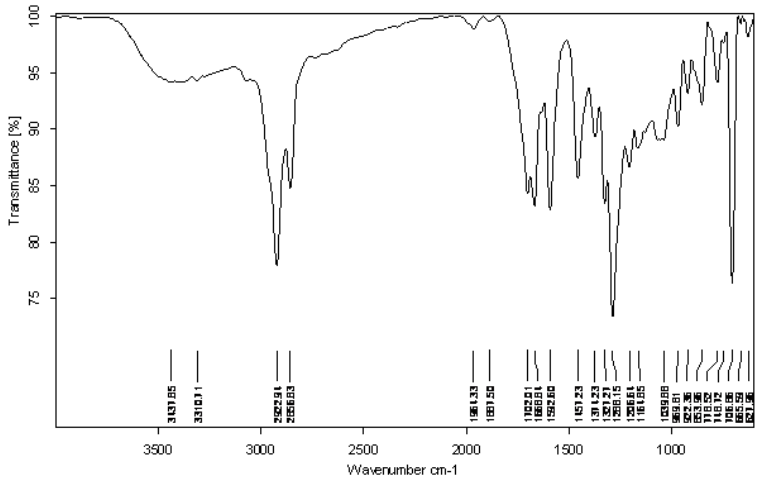


Fig. 4. FT-IR spectrum of Eicos-5-enoic acid

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