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Article

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Extraction of Medicinal Compounds From *Cynodon Dactylon* Using 1-Ethyl-3-Methylimidazolium Lactate

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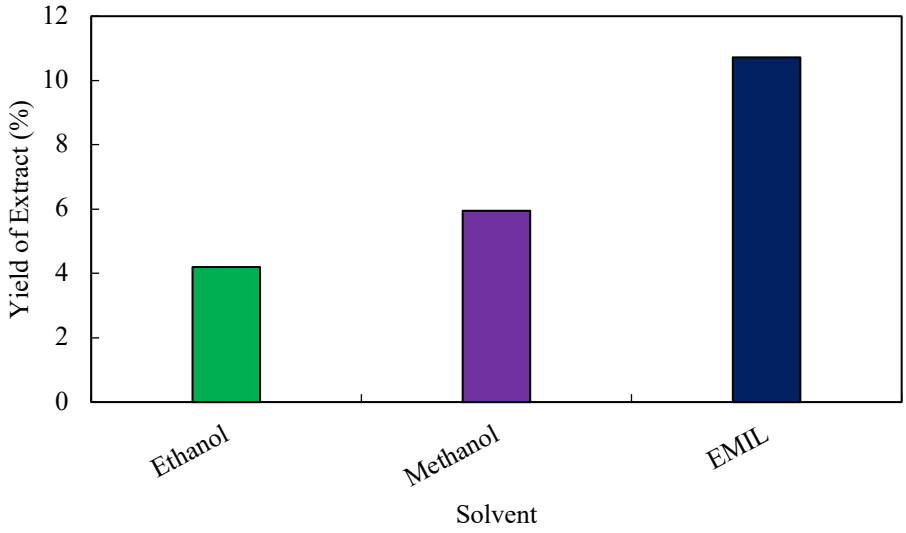
Graphical abstract



Abstract

Cynodon dactylon (CD) has been rich in metabolites and keep several biological activities. The objective of this paper is to extract medicinal compounds from CD by using green solvent, 1-ethyl-3-methylimidazolium lactate (EMIL) ionic liquid (IL) for the first time. In addition, the obtained results were compared with the extraction using organic solvent such as methanol and ethanol. The extractions using organic solvents, methanol and ethanol, were conducted in a Soxhlet Apparatus at temperatures of 65-80^{o}C and IL based extraction was carried out in a round bottle flask at a temperature of 65^{o}C and IL concentrations of 0.004-0.012 g/mL. The extract amount was analysed using volumetric method and the qualitative analysis was done using Gas Chromatography and Mass Spectrometry (GC-MS). The results show that maximum 10.72% extract was extracted at a temperature of of 65^{o}C and an IL concentration 0.012 g/mL that was approximately 80.17% higher than methanolic extraction (5.95%) and 155.24% higher than ethanolic extraction (4.20%). In addition, a significant quantity of medicinal compounds such as oleic acid and fatty acid derivatives (9-Octadecenoic acid (Z)-, methyl ester) were successfully extracted which have antioxidant, antitumor, antifungal and anticancer activity. Therefore, EMIL was active to extract medicinal compounds from CD.

Keywords**:** *Cynodon Dactylon***,** Bermuda grass, 1-ethyl-3-methylimidazolium lactate, Soxhlet Apparatus, Medicinal compound, Biological activity

1.0 INTRODUCTION

Over thousand years plants have been utilized traditionally to treat many diseases before their potential in medicine were being realized by researchers. For decades researchers have done numerous works to explore the medicinal potential from plants which help to boost the pharmaceutical development. Medicinal plants play a very important role in pharmaceuticals industry to develop alternative drugs to overcome the pitfalls possessed by the synthetic drugs. Chemical compounds in plants mediate their effects on the human body by binding to receptor molecules present in the body. Such processes are identical to those already well understood for conventional drugs and as such herbal medicine do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be in principle just as effective as conventional medicines but also gives them the same potential to cause harmful side effects. Among the 120 active compounds recently isolated from the higher plants and broadly used in modern medicine today, 80% of them show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are obtained. More than two thirds of the world's plant species, at least 35,000 of which are estimated to have medicinal value that come from the developing countries. At least 7,000 medicinal compounds in the modern pharmacopoeia are obtained from plants. Different parts such as leaf, root, stem, fruit, seed and park are used to obtain several phytochemical constituents. In addition, medicinal plants are rich in biologically active compounds and play an important role in drug discovery. The plant extracts are also being used for treatment of cancer [1]. Several anticancer agents from plants include taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, and etoposide derived from epipodo phyllotoxin are in clinical use all over the world. Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with current chemotherapeutic agents.

The weed, *Cynodon dactylon* (CD), is commonly known as Bermuda grass, Bahama grass, devil's grass or couch grass. This weed has been found to possess various potential medicinal properties, like, antidiabetic, antioxidant, and so on [2]. Duke (1983) [3] revealed that the species of CD is originated from East Africa. Now-a-days, it is found all over the world. It is especially abundant in moderate and subtropical regions. This grass is widely used in golf courses and lawns as turf grass [4]. In Bangladesh, it is not only available at public areas such as roadsides but also present in the crop-fields as a weed. It is reported in open literature that this weed is widely used as folk medications, especially in Indian culture, for curing different ailments like, cough, cramps, diarrhoea, epilepsy, headache, haemorrhage, hypertension, hysteria, insanity and snakebite [3]. The researchers have detected many compounds in CD extract that are summarized in **Table 1**.

Table 1 Chemical compounds detected in CD extract [2-11]

Solvent and parts of plant used	Detected Chemicals
Used solvent: Aqueous, Phosphate buffered saline, ethanolic, methanolic, phenolic fraction, aqueous and alcoholic, hydroalcoholic, chloroform-methanolic, ethylacetate fraction, acquous and non-polysaccharide fraction, butanolic	β-sitosterol, β-carotene, palmitic acid, triterpenoids, arundoin, friedelin, selenium, alkaloids- ergonovine and ergonovinine, Ferulic, syringic, p- coumaric, vanilic, p-hydroxybenzoic and o-hyroxyphenyl acetic acids, cyanogenic hyperoside, cyanogenic glucoside- triglochinin, furfural, furfural alcohol, phenyl acetaldehyde, acetic acid, phytol, β-ionone; mono and oligosaccharides, lignin; hydrocarbons (tritriacontane) esters, eicosanoic and docosanoic acids, free alcohol, free aldehydes (hexadecanal)

Solvent and parts of plant used	Detected Chemicals
Used parts: Whole plant, leaves, roots, rhizome, arial parts	and free acids (hexadecanoic acid) (surface cuticular wax); flavone – apigenin, luteolin, flavone glycosides – orientin (8-C-β-D-glycosyl luteolin), vitexin (8-C-β-D-glycosyl apigenin), iso –orientin (6-C-β-D-glycosyl luteolin) and iso-vitexin (6-C- β-D-glycosyl apigenin).

Shabi et al. in 2010 [6] have investigated a comparison study of the chemical constituents between phenolic fraction and hydro alcoholic extract of CD by GC-MS. Approximately 22 compounds are found in CD, but the yield of phenolic fraction is only 0.6%. Among the 20 characterized compounds in CD, the most abundant components are Hydroquinone (69.49%), Levoglucosenone (2.72), Furfural (6.0%) etc. In the year 2011, Jananie et al. [7] have disclosed that leaves extract of CD contain Glycerin (38.49%), 9,12-octadecadienoyl acid chloride,(Z,Z)-(15.61%), Hexadecanoic acid, ,ethyl ester (9.50%), Ethyl α-d-glucopyranoside (8.42%), Linoleic acid,ethyl ester (5.32%), and Phytol (4.89%). The presence of these components are justified the use of CD to treat many aliments in folk and herbal medicine. Kaleeswaran et al. (2010) [8] has also found n-tricosane, which are reported to show antimicrobial characteristics [9], in the ethanol extract of the leave of this species. Abdullah et al. in 2012 [10] have operated a pioneering study to investigate the phytochemical constituents of the whole plant in seven different solvents, acetone, chloroform, diethyl ether, ethanol, ethyl acetate, methanol, and n-pentane. Their results confirmed that the plant contains many bioactive compounds such as alkaloids, cardiac glycosides, terpenoids and steroids, saponins, phenolic compounds, flavonoids, tannins, carbohydrates and proteins. Ashokkumar et al. in 2013 [2] have also discovered that CD extract shows antibacterial and wound healing characteristics. Solanki and Nagori in 2013 [11] have detected β-sitosterol in herbal semisolid formulation of CD using the whole plant of CD. Jegajeevanram et al. in 2014 [12] have investigated the phytochemical composition of CD leaves by GC-MS analysis for the presence of insecticidal compounds. They have concluded that the active compounds in the plant could be developed into consistently effective pesticides with additional research into triterpenoid chemistry and entomology. Therefore, the researchers have found alkaloids, flavonoids, steroids, triterpenoids, tannins, phenols, glycosides and so on from various part of the plant such as rhizomes, leaves, root, stem and also the whole plant using variety of solvents such as acetone, methanol, ethanol, water and etc. **Table 2** illustrates the medicinal values of the extract of CD.

Table 2 Medicinal values of the most common constituents found in CD [2]

Constituents	Medicinal Value(s)
Alkaloids	Cough medicine, antiarrhythmic, antihypertensive, antitumor
Flavonoids	Anti-viral, anti-cancer, anti-inflammatory, anti-allergic, antioxidant
Steroids	Inflammation treatment
Triterpenoids	Cancer treatment, antioxidants, antibacterial, analgesic
Tannins	Antioxidant, antimicrobial
Phenols	Anticancer, anaesthetic/analgesic
Glycosides	Analgesic, anti-rheumatic, anticancer, anti-inflammatory

It is concluded from the above literature review that CD extract contains a lot of medicinal compounds as well as bioactive compounds. But the previous studies were done using harmful organic solvents. Current study focuses on green solvent. Capello et al. in 2007 [13] defined the main objective of green solvents as to minimize the effect of using solvents in the chemical processing towards the environment.

Current world dislikes organic solvents. Water (H_2O), an inorganic compound, is recognized as a green solvent as it is safe and environmentally benign solvent [14]. But this solvent cannot give maximum yield in all extraction processes. Hence, the researchers have studied on other alternatives like Ionic Liquids (ILs), an organic salt having low volatility and flammability. ILs are also used as green solvent in some extraction processes. In 2000, Earle and Seddon [15] have disclosed that ILs can be used not only as designer solvents, but also as a reaction medium in different type of reactions. It is reported in open literature that some ILs can produce toxic or hazardous by-products [16]. Therefore, the selection of proper IL is vital for environmental and safety point of view. Open literature revealed that 1-ethyl-3-methylimidazolium lactate (EMIL) is an environmentally benign IL.

The objective of this paper was to extract medicinal compounds from CD by using EMIL IL and the obtained results were compared with the solvent extraction such as methanol and ethanol.

2.0 METHODOLOGY

2.1 Sample Collection and Preparation

The whole body of CD was collected discretely from different places of Singair, Manikganj in rainy season. The collected plants were cleaned properly with water to remove soils, dirt and unwanted parts of the plants. The specimens were then dried overnight in an oven at a temperature of 60^{o}C. The dried plants were pulverized using an electrical grinder to form powder to increase the surface area for extraction. The particles of CD in a diameter of 250 μm or less were separated through sieve analysis and were used as samples for extraction. The samples were kept in a refrigerator to use it during experiments.

2.2 Extraction Procedure

2.2.1 Ethanolic or methanolic extract

Exactly 250 mL of ethanol or methanol was taken in a 300 mL round bottle flask. Then, approximately 5 g of CD sample was placed in a pre-measured thimble and the thimble was loaded in the Soxhlet apparatus. The extraction was performed for 8 hours at a temperature of 80^{o}C (for ethanol) or 65^{o}C (for methanol). The extract was then collected in sample bottle and the samples were stored in refrigerator until further use. The thimble was removed from the apparatus and was dried in an oven. Finally, the exacted amount was calculated by measuring the dried thimble. The experiments were done three times with 95% confidence and the average values are reported.

2.2.2 IL-based extract

Exactly 250 mL of methanol was placed in a 300 mL round bottle flask. Then exactly 1 - 3 g of EMIL was added into the round bottle flask to make its concentration 0.004 – 0.012 g/mL. Approximately 5 g of CD sample was placed in a pre-measured thimble and the open-end of the thimble was closed with cotton. The thimble was then placed into the round bottle flask. A condenser was kept at the top of the round bottle flask to prevent vaporization of volatile compounds. The round bottle flask was then heated at a constant temperature of 65^{o}C and a time of 8 hr. The thimble was removed from the round bottle flask, was cleaned with methanol and was dried in an oven. Finally, the extracted amount was quantified by measuring the dried thimble.

2.3 Extract Analysis

The amount of extract was analysed using gravimetric methods. The percentage of extract was then calculated using the following formula:

$$Yield\ of\ extraction\ (\%) = \frac{A-B}{A} \times 100\ \% \qquad (1)$$

where A = Loaded amount of CD in g in the extractor and B = Amount of CD in g after extraction

GC-MS analysis was carried out to identify the compound present in the extracted samples. The GC-MS analysis was conducted on Agilent Technologies 7890A Gas Chromatograph with 5975C Mass Spectrometer using 30 m length, 0.25 mm diameter and 0.25 μm DB-23 column. Exactly 1µl extract was injected into the GC-MS using micro syringe at split less mode at temperature of 200ºC. The column oven temperature was hold at 45°C for 1 minute, then increased at a rate of 5°C/ min to reach 280°C and then hold for 15 min. Helium gas was used as a carrier gas at a flow rate of 1.4 mL/min. Total GC running time was 48 min.

3.0 RESULTS AND DISCUSSION

To investigate the solvent effect, three different solvent such as ethanol of boiling point 78.37ºC, methanol of boiling point 64.70ºC, EMIL at different concentrations in methanol were used. The ethanolic and the methanolic extraction were carried out in a Soxhlet Apparatus at temperatures of 80ºC and 65ºC respectively and IL based extraction was carried out in a round bottle flask at a temperature of 65ºC. **Figure 1** reveals that EMIL at a concentration of 0.012 g/mL extracted maximum 10.72% extract whereas the respective ethanolic and methanolic extract yields were 4.20% and 5.95% only. Therefore, EMIL at a concentration of 0.012 g/mL extracted approximately 80.17% higher than methanolic extraction and 155.24% higher than ethanolic extraction. Abdullah et al. [10] have conducted experiments using CD collected from Universiti Malaysia Sabah's area and revealed that ethanolic extraction produced the highest yield (7.07%) followed by methanolic extract (5.42%). Therefore, the whole body of CD obtained from Singair, Manikgang areas gives almost the same amount of methanolic extract as CD of Universiti Malaysia Sabah's area.

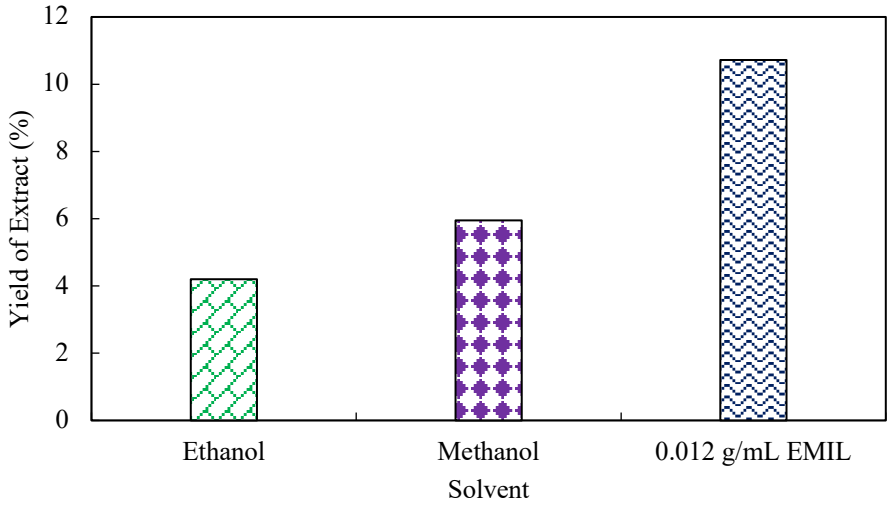


Figure 1 Extract yields obtained by using different solvents

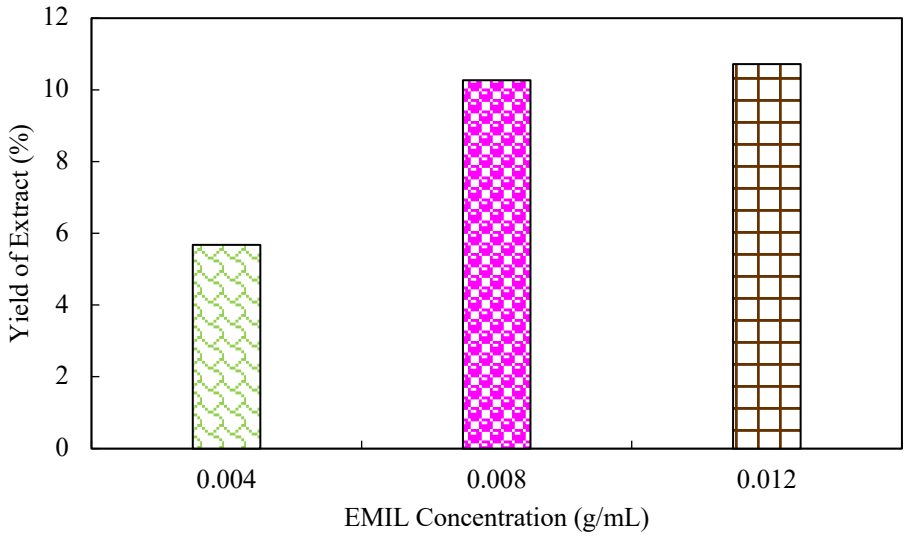


Figure 2 Extract yields at different concentration of EMIL in methanol

Figure 2 demonstrates that extract yields were increasing with the increase of concentration of EMIL in methanol. The yield of extract was only 5.68% at a concentration of 0.004 g/mL and this value was increased to 10.72% at a concentration of 0.012 g/mL. An escalation of yields was observed from EMIL concentration of 0.004 g/mL to 0.008 g/mL EMIL while there was only a small rise as it was further increased to 0.012 g/mL. This result indicates that the optimum extraction was very close to 10.72%. Wang et al. have disclosed that the density (1.1548-1.1140 $g.cm^{-3}$) and surface tension (53.37-48.63 $mN.m^{-1}$) of EMIL increased with the increase of temperatures of 10-60^{o}C. In addition, alike the alcohol EMIL is a hydrophilic IL. Mandal et al. in 2015 [17] revealed that IL molecules themselves could form cage structure through specific chemical bonds. In this cage, the extract molecules would be captured through forming liquid clathrate. These characteristics of IL increased its solubility power to extract more compounds than methanol.

The GC-MS analysis detected many phytochemical compounds in the extract of CD. The extracted phytochemical compounds and their corresponding biological activities are depicted in **Table 3**. It is seen that antitumor and anticancer active compound oleic acid found in both ethanolic and EMIL (0.012 g/mL) based extract. In addition, Antifungal, antioxidant, anticancer active compound 9-Octadecenoic acid (Z)-, methyl ester was found only in EMIL (0.012 g/mL) based extract. Furthermore, Octadecanoic acid can be used as Cancer preventive insectifuge that was found in both ethanolic and EMIL (0.004 g/mL) based extract. Therefore, EMIL based extraction can extract more medicinal compounds that ethanolic and methanolic extraction.

An organosilicon compound dimethoxydimethylsilane was observed in all the extract. Kregiel and Niedzielska in 2014 [18] disclosed that this compound can greatly improve the antiadhesive and antibacterial properties of a chemically altered polyethylene. In addition, organoborate compound boric acid, trimethyl ester, commonly known as trimethyl borate, was also extracted in all extract except ethanolic extract. This compound is used to synthesize barbigerone analogues that have high anti-proliferative behaviour [19].

Table 3 Major phytochemical component detected by GC-MS equipment

Component Extracted	Peak Area (%)				Biological activity	Reference s
	Pure ethanol	Pure methanol	EMIL concentration in methanol (g/mL)			
			0.004	0.012		
Silane, dimethoxydimethyl-	2.80	19.34	16.34	1.52	Not reported in open literature	
Boric acid, trimethyl ester		16.15	13.75	2.09	No activity reported	[20]
Oleic Acid	1.50			1.02	Antitumor, anticancer	[21, 22]
1,2-Benzenedicarboxylic acid, dicyclohexyl ester	1.30				Not reported in open literature	
1,3,12-Nonadecatriene		1.41			Not reported in open literature	
1,9-Tetradecadiene				1.10	No activity reported	[23]
2-Methyl-2-docosene			1.14		Not reported in open literature	
Octadecanoic acid	2.12		2.23		Cancer preventive insectifuge	[22]
6-Octadecenoic acid, (Z)-	1.58		1.20		Not reported in open literature	
9-Octadecenoic acid, (E)-	1.37				Not reported in open literature	
9-Octadecenoic acid (Z)-, methyl ester				2.12	Antifungal, antioxidant, anticancer	[22]
Propanenitrile, 3-[2-(4-pyridyl)-1-indolyl)-	1.28				Not reported in open literature	
cis-11-Hexadecenal	1.14				Not reported in open literature	
E-9-Hexadecenal		2.87			Not reported in open literature	
Cyclopropaneoctanal, 2-octyl-	3.40				Not reported in open literature	
Octadecanal, 2-bromo-			2.52		Not reported in open literature	
Methyl 3-hydroxyoctadec-9-enoate			3.09		Not reported in open literature	
Z,Z-6,24-Tritriacontadien-2-one			4.42		Not reported in open literature	
Cyclohexadecane, 1,2-diethyl-			1.21		Not reported in open literature	
9,12-Octadecadien-1-ol, (Z,Z)-				14.46	No activity reported	[24]
Z,E-3,13-Octadecadien-1-ol	3.81				Not reported in open literature	
2-Methyl-Z,Z-3,13-octadecadienol				2.34	Not reported in open literature	

4.0 CONCLUSION

The medicinal plant, CD, obtained from Singair, Manikganj, Bangladesh contains antioxidant, antitumor, anticancer and antifungal active compounds. EMIL extract at a concentration of 0.012 g/mL yields respective 80.17% and 155.24% higher extract of ethanolic and methanolic extraction. Moreover, extract yield was increased with increasing the concentration of EMIL in methanol from 5.68% to 10.72%. In addition, EMIL can extract more medicinal compounds such as oleic acid, 9-octadecenoic acid (Z)-, methyl ester compare to ethanolic and methanolic extract. Other extracted compounds such as dimethoxydimethylsilane and trimethyl borate have their respective application in chemical industries. Extensive study related to extract optimization and kinetics will be required to discover the potentiality of commercial production.

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