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Screening of Bioactive Compounds of Costus And Cidir Using Gas Chromatography-Mass Spectrometry.

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ABSTRACT

The medicinal effect of each of Cidir (*Zizyphus spina-Christi*) and Costus (*Saussurea lappa*) was used as traditional medicine in Saudi Arabia.The present study Cidir (Zizyphus spina-Christi) and Costus (Saussurea lappa) plants were analyzed for the chemical molecules. The best effective fraction were utilizing and identified as quantitative phytochemic alanalyses using gas chromatography-mass spectrometry (GC/MS) analytical methods, compare by database library mass spectra of the GC/MS identified compounds with those in the National Research center (NRC). The chemical analysis evidenced the presence of active compounds such as glycosides, saponin, tannins, phenols, terpenoids, alkaloid, steroids, and flavonoids in the aqueous extract of Costus and Cidir. Therefore, Cidir and Costus could be contemplating as a power source of herbal formula and pharmaceutical drug therapy product.

Keywords: Costus, Cidir, GC/MS; phytochemical analysis

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INTRODUCTION

Saussurea lappa is described as dark brown or grey, robust, pubescent, erect, perennial herb, roots are stout, up to 40 cm long (Alnahdi et al 2017). Costus or Kuthroot is common name for(*Saussurea lappa Clarke*)(Barakat et al., 1993). There are prime active molecules are dehydrocostus lactone, costic, alantolactone, palmitic, β-sitosterol, Costunolide, isoalantolactone, cyclocostunolide, linoleic acids, and cyclocostunolide (Prakash 2014), which have medicinal effects as Immunomodulatory, Hypoglycaemic Anti-inflammatory, Antimicrobial, Hypolipidaemic and Antiparasitic (Shanmugam et al., 2010). *Saussurea lappa* was utilized as a part of the dental treatment sicknesses as it is viable against caries-prompting Streptococcus mutans (Ramya and Daniel 2012).Ethanolic concentrate of the underlying foundations in *Saussurea lappa* has antioxidant activity agent movement out of suppression of nitric oxide generation in lipopolysaccharide. The isolated molecules and their mixture showed antimicrobial effect.

Zizyphus spina-christi, the Arabs calls it Nabka or cidir. Cidir fundamental oil contains 11.5% linalool and 16.4%alpha-terpineol (Uchegbuet al 2015). The significant hydrocarbons were n-pentacosane with (81%), likewise methyl esters were found in leaves as methyl palmitate, methyl stearate and methyl myristate. Maslinic corrosive, oleanolic and β-Sitosterolcorrosive were the primary aglycones of the glycosides additionally present. Leaf sugars revealed were xylose, arabinose, rhamnose, galactose, glucose, and lactose (Mary and Syhed 2011).

The leaves of *Zizyphus spina-christi*c ontained (0.66%) Flavonoid as glycoside kaempferol 3-O-α-L-rhamnopyranoside (Uchegbuet al 2015). *Zizyphus spina-christi* was likewise answered to have free radical rummaging action. Plant leaves were utilized as a part of pharmaceutical as a calming, germ-free, and antifungal operator for infections of skin (Malinieet al 2013). The saponin part of the leaves has an antimicrobial movement against *Candida albicans* (Husam and Kredy 2010). Phytochemical investigation of the rough concentrates of the ethanol and water of the leave of the *Zizphus spina-christi* pronounced the nearness of flavonoids, terpenoids Saponins, alkaloids, tannins, steroids and glycosides, (Maluventhan and Sangu 2010).

Plants were utilized widely in conventional solution in Saudi Arabia. Therefore; to the best of our insight no endeavors have been made to recognize the substance compounds exhibit in plants Costus and Cidirutilized by local people in the treatment of a few fiery infections. Thenceforth, this paper aimed to identify its chemical compounds meant to explore the substance syntheses of wrinkle phenolic content and for the two chose plants, Saussurea lappa and Zizyphus spina-christi.

MATERIALS AND METHODS

Collection of plant materials

Two kind of medicinal plants were evaluated for phytochemical analyses. Plants were obtained from the local herbal market in Kingdom of Saudi Arabia. Voucher specimens from all plant materials were deposited at the Herbal Museum, Department of Pharmacology, Faculty of science, King Abdulazziz University of Medical Sciences, for identification. The scientific name, family name, English name, and traditional use of plants are presented in Table 1. The plants were cleaned, dried at room temperature. 50 g of the dried plant was ground well to fine powder in electrical grinder (ANEX AG-694). All plant samples were stored at 10°C until analyzed.

Table 1: Characteristics of the used medicinal plants Costus and Cidir

English name	Family name	Scientific name	Part used	Traditional indications
Costus	Asteraceae	*Saussurea lappa*	*Roots*	cough with cold, stomachache, anticancer, anti-inflammatory, cardio tonic, dysmenorrhea, analgesic, and anti-fatigue
Cidir	Rhamnaceae	*Zizyphus spina-christi*	Leaves Seeds roots	digestive disorders, weakness, liver complaints, obesity, urinary troubles, diabetes, skin infections, loss of appetite, fever, pharyngitis, bronchitis, anemia, diarrhea, and insomnia

Plant processing, extraction

A quantity (10 g) of each powdered plants was soaked in 100 ml of hot water (70°C) in a shaker at room temperature for 1h stirring. The extracts were filtered using Whatman No.1 filter paper and the filtrates were subsequently concentrated using rotary evaporator at 30°C (Buchi Rotavapor RE; Switzerland). The concentrates obtained were reconstituted in water (1:10 w/v).Samples were stored until used at 10°C until analyzed.

Phytochemical Screening

Phytochemical screening was carried out for analyzing the secondary metabolites which are responsible for curing various human aliments. The phytochemical screenings of the extract were accessed by the standard method (Shanmugam et al., 2010) to detect the presence or absence of certain bioactive compounds. Water extracts were used to identify the major natural chemical compounds such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides and steroids.

Gas chromatography-mass spectrometry (GC/MS)analysis

The GC-MS Specification was: Agilent Technologies model 7890A GC-MS, MSD=5975C (detector) Agilent Technologies, Injector: 7683B series, initial temperature=100 °C held for 2 min, final temperature=270 °C at the rate of 10°C/min, 1 µL of 0.2 g/mL fraction was injected. Temperature of heater was 250°C, pressure was 3.2652psi, mode type splitless, column type (HP5MS: 30 mol/L×320 µmol/ L×0.25 µmol/L) and carrier gas (helium, 99.999 9% purity, flow rate=1.4963 mL/min; average velocity=45.618 cm/sec). The constituent compounds were determined by comparing the retention times and mass spectrum of the authentic samples obtained by GC with the mass spectra from the National Research center (NRC) Version 2.0 MS database library.

Identification of Components

Interpretation of mass spectrum of GC-MS was done using the database of National Research center (NRC) having more than 62000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NRC library. The name, molecular weight and structure of the component of the test materials were identified.

RESULTS AND DISCUSSION

Phytochemical screening

In this study, data showed that plants usage as traditional plant in Saudi Arabia analysis. The phytochemical examination was outright for aqueous extract of *Zizyphus spina-christi* and *Saussurea lappa*. The results were presented in the Table 2,3 showed that, aqueous extract of Costus and cider were found to be extensive in terpenoids, Quinones, Phenols, alkaloids, saponins, glycosides, steroids, tannins and flavonoids respectively. This is in agreement with the statement made by (Anubha Arora, 2013) that the phytochemical screening demonstrated the presence of different types of phyto compounds like alkaloids, saponins, flavonids, steroids, tannins etc which could be responsible for the various pharmacological properties. Phytochemical constituents, for example, tannins, flavonoids, alkaloids and a few other secondary metabolites compounds of plants fill in as safeguard component against predation by numerous of microorganism, herbivores and insects. The therapeutic characteristic of medicinal plants are maybe religion to the existence of different secondary metabolites Like phenols, flavonoids, glycosides, saponins, alkaloids etc (Anubha Arora, 2013Saponins characteristic inclination to avoid microbes makes them great contender for treating yeast and fungal infections. These mixes filled in as normal anti-infection agents, which help the body to battle diseases and microbial intrusion (Santhi *et al*., 2011). Flavanoids have great biological functions as apart from its antioxidant properties include the prevents against free radicals, microbes, inflammation, allergies, platelet aggregation, ulcers, hepatoxins, viruses and tumors (Barakat et al., 1993). Cardiac glycosides content was found in methanol extract. Cardiac glycosides have been utilized for through two horns as stimulant in case of cardiac failure (Trease and Evans, 1998).

The density of flavonoids in different, zizyphus extracts was analyses using aluminum chloride method with spectrophotometric. Flavonoids content were uttered in provision of rutin equivalent and it is the highest in methanolic extracts because of the polarity of methanol. Due to the fact that plant extracts commonly present as a collection of different kinds of active compounds or throw different polarities, their division still remains a big defy of bioactive. The main chemical molecules of interest in the previously reports have been steroidal sapogenins and alkaloids (saponins) as though; another different groups of course in process phytochemicals like tannins, triterpenoids, flavonoids, unsaturated sterols, essential oils etc. also have been reported (Farnsworth *et al*., 1966).

Furthermore, the study of Quantitative phytochemical fixed a perceivable, contain of flavonols, flavonoids, proanthocyanidins, tannins, polyphenols, saponins and alkaloids. In present study, the screening phytochemical of Cidir and Costus eques extract showed that, high level in Quinones, Tannins, Terpenoids, Flavonoids, Saponins, Glycosides, Phenols, Steroids and Alkaloids. Phytochemical constituents such as alkaloids, flavonoids, tannins and several other aromatic molecules are secondary metabolites that serve as defense mechanism against different kind of microorganisms (Mary and Syhed 2011). Flavonoids are the most water soluble antioxidants which prevent oxidative cell damage and possess anticancer found to have anti-bacterial, antiulcer and antiviral properties (Subashri and Justin 2014).

GC – MS analysis of the plant extract has established 10 bioactive compounds which possess several pharmacological properties. Phytochemical screening and GC-MC studies confirm presence of phenolic compound mainly responsible for the antimicrobial property of the plant. The present study unveil the medicinal important of bioactive compounds present in the ethanol extract of *Costus sand Cidir*. The antibacterial properties of the extract may be due to the presence of above mentioned phytochemicals.

Table 2: Phytochemical screening of Costus

Peak	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.289	VB	0.1458	953.35577	34.0318	?
2	6.157		0.0000	0.00000	0.0000	Gallic acid
3	10.860		0.0000	0.00000	0.0000	Catechin
4	12.121	BV	0.1513	246.12695	8.7860	?
5	14.215		0.0000	0.00000	0.0000	Coffeic acid
6	14.752		0.0000	0.00000	0.0000	Syringic acid
7	15.543		0.0000	0.00000	0.0000	Rutin
8	17.248	VB	0.1571	33.24746	1.1868	Coumaric acid
9	18.048	BV	0.1172	62.27669	2.2231	?
10	18.267	VB	0.1154	76.19844	2.7200	?
11	19.578	BB	0.1696	233.17601	8.3237	Vanillin
12	20.493	BV	0.1905	202.84427	7.2409	?
13	20.802	VV	0.2503	309.36606	11.0434	?
14	21.190	VB	0.3964	684.77411	24.4443	Querectin
15	21.410		0.0000	0.00000	0.0000	Cinnamic acid
Totals :				2801.36576		

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Table 3: phytochemical screening of Cidir

Peak	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.440	VV	0.1145	799.87817	20.9801	?
2	6.157		0.0000	0.00000	0.0000	Gallic acid
3	10.860		0.0000	0.00000	0.0000	Catechin
4	14.215		0.0000	0.00000	0.0000	Coffeic acid
5	14.697	BV	0.1449	80.48204	2.1110	Syringic acid
6	15.543		0.0000	0.00000	0.0000	Rutin
7	16.464	BV	0.1519	125.93502	3.3032	?
8	16.715	VB	0.1471	383.89169	10.0692	?
9	17.324	BB	0.1297	43.74781	1.1475	Coumaric acid
10	18.056	VB	0.1740	115.89252	3.0398	?
11	18.688	VV	0.1309	801.61316	21.0256	?
12	19.709	BV	0.1231	484.91782	12.7190	Vanillin
13	20.294	BB	0.0928	602.11011	15.7928	?
14	21.085	MM	0.2412	374.08344	9.8119	Querectin
15	21.410		0.0000	0.00000	0.0000	Cinnamic acid
Totals :				3812.55177		

GC-MS Analysis

Gas Chromatography –Mass Spectrometry is a potent tool for identifying the bioactive compounds present in the natural product. The GC-MS chromatogram of Costus and cider showed nine and ten peaks respectively indicating the presence of chemical constituents. The synthetic constituents were described and recognized on examination of the mass spectra of the constituents with the NRC library. The active molecules with their molecular formula, molecular, weight retention time and peak area (%) are presented in the Table 4,5. GC-MS analysis revealed the presence of active metabolites comprising of alkaloids, fatty acids, methylesters, alcohols, terpenoids and heterocyclic compounds.

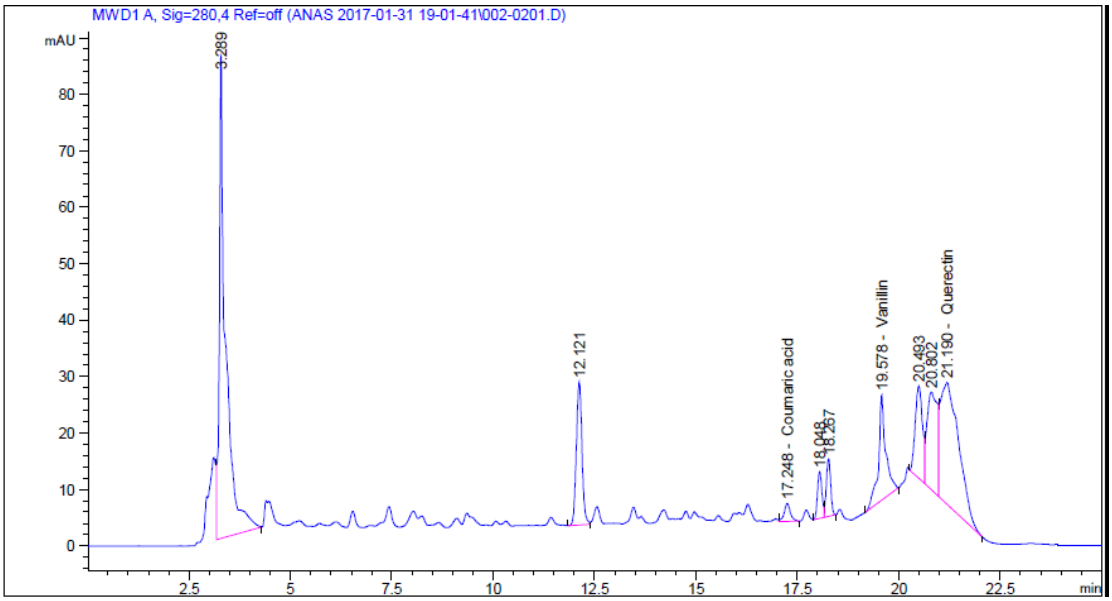

Figure 1: GC/MS chromatogram of leaf *Saussurea lappa*

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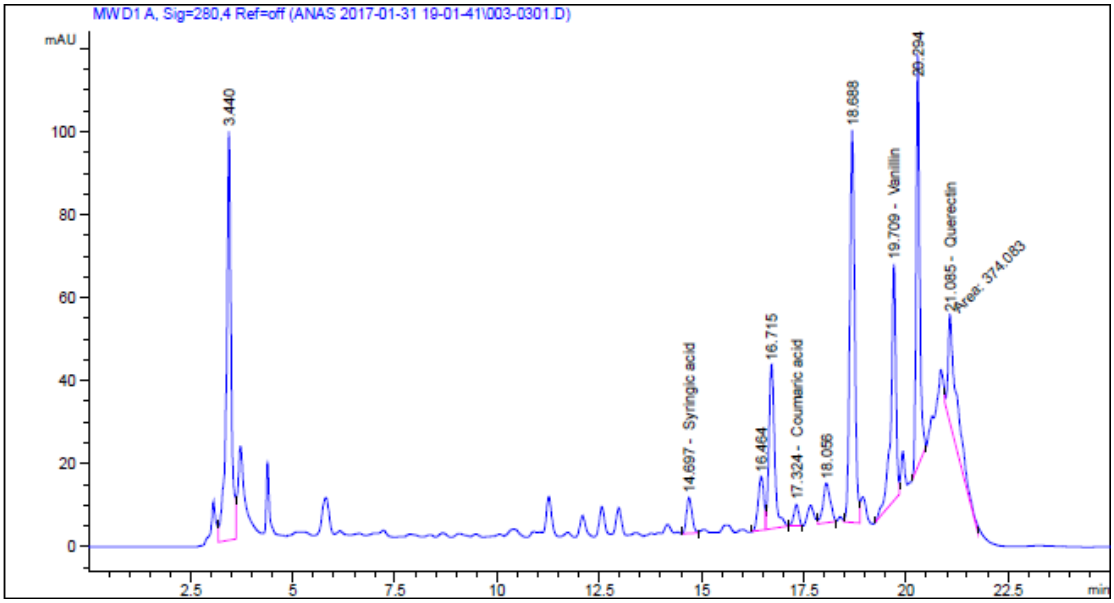

Figure 2: GC/MS chromatogram of *Zizyphus spina-Christi*

Phenolic compounds

Phenolic compound profiles of aqueous extract for Costus and cider were shown in Table 2 and 3.Six phenolic acids (gallic, caffeicaci, syringic, coumaric, vanillin and cinnamic), and 3 flavonoids (catechin, Querectin and rutin) were identified as standard. Querectin was particular as the predominant phenolic compound ranging from 9.8 to 24.4 mg/100 g DW for cider and Costus respectively. Ciderdis play best retention of phenolics than Costus in the present of syringic acid with 2,1 mg/100 g DW. Coumaricacid was fond in the same low concentrations for both Costus and cider 1.1 mg/100 g DW. Vanillin was identified as phenolic compound in, ranging from 8.3 to 12.7 mg/100 g DW for costus and cider respectively. Quantitative phytochemical ponder showed a considerable measure of alkaloids, tannins, polyphenols, proanthocyanidins, flavonoids, and flavonols saponins.

Furthermore, flavonoids and saponins have been reported to exert profound stabilizing effect on lysosomal membrane while saponins and tannins bind cations, thereby stabilizing erythrocyte membranes and other bio compound (Amin et al 2013). Alkaloids are known anti-inflammatory effects. Phenolic and Flavonoid compounds are most antioxidants which prevent oxidative cell damage and also possess anti-inflammatory, anti-thrombotic and anti-allergic (Uchegbu et al 2015). Proanthocyanidins are a kind of flavonoid that has been shown to have completely, effective antioxidant activity (Savithramma et al 2011). Past investigations had additionally demonstrated that plant extricates having mitigating properties may contain phytochemicals with cell reinforcement movement against antioxidant responses activated by receptive oxygen species related with irritations(Alnahdi et al 2017). Tannins contribute property of astringency i.e. speedier the mending of wounds and aroused mucous membrane (Okwu and Josiah, 2006). It should be prominent that steroidal compounds are of significance in pharmacy due to their correlation with sexal hormones (Anubha Arora, 2013). Phenols are essential plant in light of their searching capacity on free radicals because of their hydroxyl gatherings, thusly, the phenolic substance of plants may contribute specifically to their antioxidant activity (Garcia et al 2006). Therefore, the phenolic content of plants may share in immediately to their antioxidant activity (Alnahdi et al 2017) .

CONCLUSION

The medicinal plant screened was found to be high in secondary metabolites which are used in traditional medicine to combat and cure various diseases. Phytochemical analysis showed that biological activity of Costus and Cidir was due to the presence of phytochemical such as Phenols, Saponins and Tannins. Thus these plants can be utilized as a useful source to formulate new antimicrobial drugs of natural origin. Further studies are needed to isolate and characterize the structure of bio active compounds for drug

formulation. The present study leads to the further research in the way of isolation and identification of the activity compound from the selected plants using chromatographic and spectroscopic techniques.

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