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Qualitative phytochemical screening of some selected medicinal desert species

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Abstract
Desert plants are considered as a rich source of secondary metabolites and are used in drug development. The main objective of the current investigation was to explore the secondary metabolite profile of some desert plants and also compare the efficiency of different solvents. Plants [*Psidium guajava* L., *Thuja orientalis* L., *Callistemon citrinus* (Curtis) Skeels, *Capparis spinosa* L. and *Dipterygium glaucum* Decne.] were collected from the Cholistan desert of Bahawalpur based on the traditional knowledge. Five types of extracts (petroleum ether extract, benzene extract, chloroform extract, ethanol extract and methanol extract) were prepared for both fresh and dry plant materials. The methanol extract showed the highest efficiency to detect the secondary metabolites as compared to that of all other extracts. Qualitative phytochemical analysis of these plants confirmed the presence of various important secondary metabolites, viz. phlobatannins, alkaloids, steroids, glycosides, flavonoids, terpenoids, quinones, anthocyanins, saponins, phenols, phytosterols and tannins in different parts (leaf, fruit, seed and whole plant). These results clearly show that potential bioactive compounds can be effectively extracted from important medicinal plants which could be potentially utilized by pharmaceutical industries.

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Introduction

Secondary metabolites occur widely in plants and they are known to have a multitude of biological effects, as they can be used as antifungal, antiviral, and antibiotic (Osbourn et al., 2003). They are also very common in desert plants, that is why they are used in traditional medicines by the inhabitants of deserts (Tabuti et al., 2003). According to WHO, about 80% of the world's population treats ailments with herbal medicines and 25% modern drugs derived from medicinal plants (Jasuja et al., 2013). The reason for the abundance of medicinal plants in the desert of Pakistan is its geographical location, environment and soil conditions (Shafi et al., 2001). Plants can form bioactive compounds and all of these compounds are found in fruits, leaves, roots and stems (Suffredini et al., 2004; Erb and Kliebenstein, 2020), though to varying levels.

Metabolites play a very important role in plants. For example, tannin prevents the auto-oxidation of lipids (Falleh et al., 2008; Sieniawska, 2015). Also, plants are protected from herbivores due to phenolic compounds (Zucker, 1983). Flavonoids are used as antiviral and anticancer agents (Seyoum *et al*., 2006) as well as anti- allergic (Middleton et al., 2000). Terpenoids and sterols reduce the growth of tumor cells and also lower down the cholesterol level (Juan et al., 2008). In the process of respiration and photosynthesis, quinones act as primary metabolites and saponins are also known as fungicides and insecticides. The alkaloids are known by their activities as anti-inflammatory and antipyretic (Sparg et al.,

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2004). With a myriad of beneficial roles of secondary metabolites occurring in medicinal plants, the major objective of carrying out this investigation was to uncover the secondary metabolite profile in some selected commonly occurring plants in the Cholistan desert.

Materials and Methods

Plant collection

Fresh leaves, fruits, and seeds were collected from the Cholistan desert Bahawalpur (**Table 1**).

Table 1: List of plants collected from Cholistan desert

Plant scientific name	Common name	Family
Psidium guajava L.	Amrood	Myrtaceae
Thuja orientalis L.	Oriental thuja	Cupressaceae
Callistemon citrinus (Curtis)	Bottle brush	Myrtaceae
Capparis spinosa L.	Kubber	Capparidaceae
Dipterygium glaucum Decne.	Fehl	Capparidaceae

Study area

Cholistan is a desert covering an area of 26,000 km^2 located between 27° 42´ and 29° 45´ latitude and 69° 52´ and 75° 24´ E longitude at a height of 112 m above the sea level. The mean summer temperature is 34-38 °C, while that of winter is 15-20 °C. The soil of Cholistan contains a very low quantity of organic matter (Arshad et al., 2007).

Preparation of plant materials

Different plant parts such as leaf, root, fruit, seed etc. were collected from plants, washed, cut into small pieces and then dried for 10-15 days at room temperature to eliminate the moisture content. Dry plant parts were converted into fine powder using an electrical grinder (Parveen et al., 2016). Phytochemical screening was done in two forms, i.e., in dry and fresh plant parts.

Preparation of extracts for screenings

The fresh and dry materials (each 20 g) were added to 100 ml petroleum ether in a separate beaker and the mouth covered with an aluminum foil to avoid evaporation and exposure to light for 3 days at room temperature. The extract was filtered with a Whatman filter paper. The same procedure was repeated for the preparation of benzene, chloroform, methanol, and ethanol extracts.

Phytochemical screenings

Phlobatannins

Plant material (500 mg) of each dry and fresh was dissolved in dH_2O and filtered. The filtrate was mixed with 2% HCl and then boiled. Due to this procedure, red precipitation was formed that means that phlobatannins were present in the solution (Ugoh and Neiji, 2013).

Alkaloids

One mL of each extract was taken in a separate test tube, and 2-3 drops of Mayer's reagent were added to each test tube. The formation of white precipitates indicated the presence of alkaloids (Arya et al., 2014).

Steroids

Salkowski test: Five mL of each extract were mixed in 2 mL of chloroform and 3 mL of concentrated H_2SO_4. The formation of a reddish-brown layer indicated the presence of steroids (Gayathri and Kiruba, 2014).

Liebermann-Burchard test: A trace quantity of each extract was mixed in 2 ml of chloroform and 10 drops of acetic acid with a few drops of H_2SO_4 were added to it. The change of red color to a blue color and finally to green color indicated the presence of steroids (Gayathri and Kiruba, 2014).

Glycosides

Two mL of each extract and 0.4 mL of glacial acetic acid and 0.5 mL of concentrated H_2SO_4 with a trace amount of $FeCl_2$ were added in a test tube. The appearance of blue color indicated the presence of glycosides (Arya et al., 2014).

Flavonoids

To 5 mL of each extract, a few drops of NaOH solution were added. The yellow color appeared which became colorless after the addition of a few drops of diluted H_2SO_4. This process indicated the presence of flavonoids (Arya et al., 2014).

Terpenoids

Five mL of each extract and 5 mL of $CHCl_3$ were mixed with a few drops of concentrated H_2SO_4. The mixture was shaken and then allowed to stand for some time. The appearance of yellow color in the lower layer indicated the presence of terpenoids (Arya et al., 2014).

Quinones

A small quantity of the extract was treated with concentrated HCl and the appearance of green color confirmed the presence of quinones (Aparadh et al., 2014).

Anthocyanins

A trace quantity of each extract was treated with 2 mL of NaOH. The formation of blue color indicated the presence of anthocyanins (Gayathri and Kirbua, 2014).

Saponins

Two mL of each extract and 2 mL of dH_2O were shaken for 15 min. The formation of 1 cm foam layer indicated the presence of saponins (Ugoh and Neiji, 2013).

Phenols

Five mL of each extract were dissolved in dHO with 3 mL of 10% lead acetate. Dark green color of the solution confirmed the presence of phenols (Arya et al., 2014).

Phytosterols

The extract (5 mL) was mixed with 2 mL of chloroform and 3 mL of concentrated HSO. The formation of golden yellow layer indicated the presence of steroids (Gayathri and Kiruba, 2014).

Tannins

Two mL of each extract were diluted with 4 mL of dH_2O and then added 2-3 drops of 5% $FeCl_2$. The formation of green black and blue black color indicated the presence of tannins (Ugoh and Neiji, 2013).

Results and Discussion

Medicines are made from desert plants because they contain a lot of metabolites. They are regarded as a natural source of many drugs, modern medicines, food supplements and chemical units of synthetic groups. However, to test that which type of organic solvent is effective to extract phytochemicals from plants. five different types of solvents were used to extract phytochemicals such as phlobatannins, alkaloids, steroids, glycosides, flavonoids, terpenoids, quinones, anthocyanins, saponins, phenols, phytosterols and tannins, from both fresh and dry plant material. The extraction was done according to polarity (petroleum ether > benzene > chloroform > ethanol > methanol) and compared the results of the solvents. Petroleum ether showed significant results. Glycosides were absent in three plants, *Psidium guajava*, *Thuja orientalis* and *Callistemon citrinus.* Trace amounts of phenol and flavonoid contents were present in *Callistemon citrinus* and completely absent in *Psidium guajava*. The remaining metabolites were present in optimum quantity in all studied plants (**Table 2**).

Capparis spinosa contains many bioactive compounds like flavonoids, steroids, alkaloids, terpenoids, etc. (Vahid et al., 2017), that is why it is used in herbal medicines *(*Mansour et al., 2016)*.* *Psidium guajava* leaves contain a special type of oil that contains the tannins, flavonoids, terpenoids, mineral salts, etc. (Kumar et al., 2019). *Psidium guajava* leaves are used for the treatments of various ailments like diarrhea, gastric problems and dysentery because of their pharmacological activity (Uzzaman et al., 2018). *Thuja orientalis* leaves contain flavonoids, terpenoids and diterpenoids (Jasuja et al., 2013). The leaf and bark of *C. citrinus* have optimum amounts of flavonoids and terpenoids (Parveen et al., 2016).

Table 2. List of secondary metabolites present in each plant

			PEE		BE		CE		EE		ME	
Plant name	**Part used**	**Metabolite**	**F**	**D**	**F**	**D**	**F**	**D**	**F**	**D**	**F**	**D**
		Phlobatannins	+	+	+	+	-	-	+	+	+	-
		Alkaloids	+	+	+	+	+	+	+	+	+	+
		Steroids										
		Salkowski	-	-	-	+	+	-	-	-	-	+
		Liebermann- Burchard	+	+	+	+	+	+	+	+	+	+
		Glycosides	-	-	-	-	-	-	-	-	-	-
Psidium guajava L.	Leaf	Flavonoids	+	-	+	+	+	-	+	+	+	+
		Terpenoids	-	+	+	+	+	-	+	++	-	-
		Quinones	+	+	-	-	+	+	+	-	+	+
		Anthocyanins	+	-	-	-	-	-	+	+	+	-
		Saponins	+	+	+	+	+	+	-	+	+	+
		Phenols	-	-	-	-	-	-	+	-	+	-
		Phytosterols	+	+	+	+	+	+	-	++	+	+
		Phlobatannins	+	+	+	-	+	-	+	+	+	-
		Alkaloids	+	+	+	+	+	+	+	+	+	+
		Steroids										
		Salkowski	-	-	-	+	+	-	-	-	-	+
		Liebermann- Burchard	+	+	-	+	-	++	-	-	-	++
		Glycosides	-	-	-	-	-	-	-	-	-	-
Thuja. orientalis L.	Leaf	Phytosterols	+	+	+	+	+	+	++	+	+	+
		Flavonoids	-	-	+	-	-	-	-	-	-	-
		Terpenoids	+	+	+	+	+	++	+	-	+	++
		Quinones	+	+	-	+	+	+	-	+	+	+
		Anthocyanins	-	+	-	+	-	+	-	+	-	+
		Saponins	+	+	+	+	+	+	+	+	+	+
		Phenols	+	+	+	+	+	+	+	+	+	+
		Phlobatannins	+	-	-	+	+	+	+	+	+	+
		Alkaloids	+	+	+	+	+	+	+	+	+	+
		Steroids										
		Salkowski	+	+	-	+	+	+	+	+	+	+
		Liebermann- Burchard	-	-	-	-	+	+	+	+	+	+
		Glycosides	-	-	-	-	-	-	-	-	-	-
Callistemon citrinus (Curtis)	Leaf	Phytosterols	-	-	+	+	+	+	+	+	+	+
		Flavonoids	-	-	-	-	-	-	-	-	+	-
		Terpenoids	+	+	+	+	+	+	+	+	+	+
		Quinones	+	-	-	-	-	+	-	-	+	+
		Anthocyanins	-	-	-	+	+	+	-	-	-	+
		Saponins	+	+	+	+	+	+	+	+	+	+
		Phenols	-	-	-	-	-	-	+	-	+	-
		Alkaloids	+	+	+	+	-	-	-	-	+	+
		Steroids (Liebermann-Burchard)	+	+	+	+	+	+	+	+	+	+
		Glycosides	+	+	+	+	+	+	+	+	+	+
		Flavonoids	+	+	-	-	-	-	-	-	+	+
	Leaf	Terpenoids	-	-	-	-	+	+	-	-	+	+
		Quinones	+	+	+	+	+	+	+	+	+	+
		Anthocyanins	+	+	+	+	+	+	+	+	+	+
		Tannins	+	+	+	+	+	+	+	+	+	+
Capparis spinosa		Phenols	+	+	+	+	+	+	+	+	+	+
		Alkaloids	+	+	+	+	+	+	-	-	+	+
		Steroids (Liebermann-Burchard)	+	+	+	+	+	+	+	+	+	+
		Glycosides	+	+	+	+	+	+	+	+	+	+
	Fruit + Seeds	Flavonoids	-	-	-	-	-	-	+	+	+	+
		Terpenoids	-	-	-	-	-	-	+	+	+	+
		Quinones	+	+	+	+	+	+	+	+	+	+
		Anthocyanins	+	+	+	+	+	+	+	+	+	+
		Tannins	+	+	+	+	+	+	+	+	+	+
		Phenols	+	+	+	+	+	+	+	+	+	+

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			PEE		BE		CE		EE		ME	
Plant name	**Part used**	**Metabolite**	**F**	**D**	**F**	**D**	**F**	**D**	**F**	**D**	**F**	**D**
Dipterygium glaucum Decne.	Crude whole plant	Alkaloids	+	+	+	+	+	+	+	+	+	+
		Steroids (Liebermann-Burchard)	+	+	+	+	+	+	+	+	+	+
		Glycosides	+	+	+	+	+	+	+	+	+	+
		Flavonoids	+	+	+	+	+	+	+	+	+	+
		Terpenoids	+	+	+	+	+	+	+	+	-	-
		Quinones	+	+	+	+	+	+	+	+	+	+
		Anthocyanins	+	+	+	+	+	+	+	+	+	+
		Tannins	+	+	+	+	+	+	+	+	+	+
		Phenols	+	+	+	+	+	+	+	+	+	+

Legends = PEE: Petroleum ether extract; BE: Benzene ether; CE: Chloroform extract; EE: Ethanol extract; ME: Methanol extract, F: Fresh; D: Dry

Conclusion

Desert plants are a rich source of secondary metabolites which can be used in drug synthesis and development. Analysis of the plant extracts revealed the presence of phytochemicals such as tannins, saponins, phlobatannins, alkaloids, steroids, glycosides, anthocyanins, phenols, phytosterols, flavonoids, terpenoids and quinones. Methanol extract showed the highest efficiency to detect the secondary metabolites amid all other extracts. The presence of secondary metabolites signifies the importance of these desert medicinal plants as an efficient source of therapeutic agents.

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The author(s) certify that all experimental materials were handled with care during collection and experimental procedures. After completion of experiment, all materials were properly discorded to minimize any types of bio-contamination(s).

Availability of primary data and martials
As per editorial policy, experimental materials, primary data or software codes are not submitted to the publisher. These are available with corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Author's consent
All authors contributed in designing and execution of the experiment. All contributors have critically read this manuscript and agree for publishing in IJAEB.

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