



Original article

Quantitative and qualitative analysis for standardization of *Euphorbia cuneata* VahlAwaad S. Amani^{a,*}, Monerah R. Alothman^b, Yara M. Zain^c, Saleh I. Alqasoumi^d, Esraa A. Alothman^e^a Pharmacognosy Department, College of Pharmacy, Prince Sattam Bin Abdul-Aziz University, Al-Kharj, Saudi Arabia^b Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia^c M-Pharm Department, University of Bradford, Bradford, UK^d Department of Pharmacognosy, College of Pharmacy, King Saud University, Saudi Arabia^e College of Pharmacy, Prince Sattam Bin Abdul-Aziz University, Al-Kharj, Saudi Arabia

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ABSTRACT

Euphorbia cuneata Vahl very promising plant belongs to Family Euphorbiaceae The present study was carried out on the *Euphorbia cuneata* Vahl to standardize its components. Qualitative and quantitative phytochemical analysis showed variable phytochemical groups. Examination of Successive Extraction showed that there are different color, constancy, phytochemical groups and yield in each extract, the highest percentage was found in ethanol (10.7 ± 1.01) and the lowest one in ether (1.66 ± 0.31). Analysis of primary and secondary metabolites of *Euphorbia cuneata* Vahl revealed that the primary metabolites percent (carbohydrate, lipid and protein 6.25 ± 1.11 , 5.12 ± 1.40 , 7.15 ± 1.31 W/w respectively) were lower than secondary metabolites (flavonoids, phenolic and tannins 11.26 ± 1.02 , 9.15 ± 1.21 and 5.23 ± 1.29 W/w respectively). The Pharmacopoeia Constants were determined. Amino acids analysis of the arial parts reported the presence of 15 amino acids with different percentage in different types. (Total, free and protein hydrolysate.) Arginine represented the highest concentration (20.86).

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1. Introduction

One of the largest plant families present is Euphorbiaceae which contains 321 genera and 7950 species) their distribution mainly tropics but extending into the temperate region both northern and southern hemisphere. Two major areas of distribution are America and Africa (Andréa et al., 2014).

Most of the Euphorbiaceae species contain a milky or colored latex. The latex is poisonous in some species and many species contain irritant and pesticidal substances (Rahman Akter, 2013). The largest genus of this family is Euphorbia. It comprises 700 spe-

cies of trees, shrubs or herbs with acrid milky juice (Rahman Akter, 2013).

The members of this family contain anthraquinones, triterpenoids, fatty acids, epoxides and anti-tumor agents. Alkaloids are present in the form of apmorphine, pyridine, indole, quinoline and tropane type. This family is also of economic importance such as the extraction of rubber from *Hevea sapium*. Other species are of medicinal value such as castor oil isolated from *Ricinus communis* and croton oil from cascarilla bark (Andréa et al., 2014).

This genus is of great importance due to its various phytochemical constituents as phenolic compounds (Al-Jaber et al., 2011; Wu et al., 2012; Moreira et al., 2013), terpenoids (Banibrata et al., 2015; Milan and Nenad, 2014) tannins (Liu et al., 2002; Andrea and Judit, 2014). It is also well known due to its important medicinal uses (Julius and Patrick, 2011; Awaad et al., 2013) for example acetyl choline-like action with muscarinic and nicotinic activities on isolated ileum of rabbit (Ayatollahi et al., 2010), spasmolytic (Pounikar et al., 2013), diuretic (Milan and Nenad, 2014), increase capillary strength (Pounikar et al., 2013), antileukemic (Amir, 2006), anti-inflammatory (Sener, 2013; Sun and Liu, 2011), analgesic and decrease the release of prostaglandin. Other researches

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noticed irritant and pro-inflammatory effect (Ursula and Jack, 2006; Takuo and Hideyuki, 2011).

The plant under study is *Euphorbia cuneata* Vahl growing in Desert of Saudi Arabia near red sea coast. In the course of searching for a natural curing agents, the authors did not find reported data about percentage constituents of this species, so the present work was carried out to standardize its components in order to help with its common use as medicinal plant.

2. Material & methods

2.1. Plant materials

The aerial parts of *Euphorbia cuneata*, Vahl (Euphorbiaceae) were collected during flowering stage in 2010, from Desert of Saudi Arabia. The sample was identified by Dr. Jacob T. Pandalayil (Assistant Professor of Plant Taxonomy, Botany and Microbiology Department, Faculty of science, King Saud University) and comparison with the published plant description (Migahid, 1996). A voucher specimen (KSU. NO. 6113) was deposited in the herbarium of Chemistry Department, Faculty of Sciences, King Saud University. The plant material was air-dried in the shade, reduced to fine powder, packed in tightly closed containers and stored for phytochemical and biological studies.

2.1.1. Phytochemical standardization analysis

2.1.1.1. Qualitative phytochemical analysis. The air dried powders of the plant under investigation (*Euphorbia cuneata*, Vahl.) was subjected to phytochemical screening for its different constituents according to the standard methods by Khan et al. (2011). The results of phytochemical screening are recorded in Table 1.

2.1.1.2. Quantitative phytochemical analysis.

2.1.1.2.1. Successive Extraction and percentage yield of extractives. Air dried powdered sample (100 g) was successively extracted by petroleum ether (60–80 °C) anhydrous ether, chloroform, ethyl acetate, ethanol (95%) and 50% ethanol using continuous extraction apparatus (soxhlet). Each extract was evaporated under reduced pressure at temperature not exceeding 35 °C. The percentage yield was calculated on respect to dry weight. The different successive crude extractives were subjected to physical and chemical examinations.

2.1.1.2.2. Determination of certain pharmacopoeial constants. The determination of moisture, total ash, acid insoluble ash and water soluble ash was carried out on the air dried plant powder according to Published (Alfy et al. (2012))

2.1.1.2.3. Estimation of primary and secondary metabolites percentage. Quantitative analysis of carbohydrates (Santhi and Sengottuve, 2016), proteins (Santhi and Sengottuve, 2016), lipids (Verma et al., 2013), phenols Santhi and Sengottuve, 2016), flavo-

noids (Krishnaiah et al., 2009), and tannins (Krishnaiah et al., 2009).

3. Results and discussion

The present study was carried out on the *Euphorbia cuneata* Vahl to standards its components, it revealed the presence of different active phytochemical constituents, these constituents were qualitatively and quantitatively analyzed using different analytical and spectroscopic methods, the results are mentioned in Tables 1–5.

3.1. Qualitative phytochemical analysis

The phytochemical screening of leaf, stem and flower of *Euphorbia cuneata* Vahl showed that this plant contains; carbohydrates and / or glycosides, flavonoids, sterols and / or triterpenes, protein and / or amino acids and tannins are present. On the other hand alkaloids and / or nitrogenous bases, cardinolides, saponins, anthraquinones and oxidase enzyme were absent (Table 1). The variations in phytochemical contents of the plant are due to number of environmental factors such as climate, altitude, rainfall etc. (Kokate et al., 2004).

Examination of Successive Extraction showed that there are different colors and constancy Also it recorded the Presence of sterols and / or triterpenes in petroleum ether and ether extractives. While carbohydrates and / or glycosides were detected in ethyl acetate, butanol and ethanol extracts. On the other hand Flavonoids were detected in all extractives except petroleum ether. Tannins were found in ethyl acetate, butanol and ethanol extractives. Anthraquinones, cardinolides, saponins and alkaloids and / or nitrogenous bases were absent in all extracts.

These variation of phytochemical constituents of the plant under investigation seemed to be the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital role for good health (Bhumi and Savithramma, 2014).

3.2. Quantitative phytochemical analysis

Quantitative examination of successive Extracts showed different concentrations (Table 2), the highest percentage was represented in ethanol (10.7 ± 1.01) and the lowest one in ether (1.66 ± 0.31).

Analysis of primary and secondary metabolites of *Euphorbia cuneata* Vahl (Table 3) revealed that the primary metabolites percent (carbohydrate, lipid and protein 6.25 ± 1.11 , 5.12 ± 1.40 , 7.15 ± 1.31 W/w respectively) were lower than secondary metabolites (flavonoids, phenolic and tannins 11.26 ± 1.02 , 9.15 ± 1.21 and 5.23 ± 1.29 W/w respectively). The presence of high

Table 1
Qualitative phytochemical analysis of *Euphorbia cuneata* Vahl.

Parameters	Petroleum ether	ether	chloroform	Ethyl acetate	Butanol	95 % ethanol
i. Physical examination	s.s &g.	s.s &g.	s.&b.	s.&b.	s.s &d.b.	s.s &d.b.
ii. phytochemical screening						
1. Sterols and/or triterpenes	+	+	–	–	–	–
2. Cardinolides	–	–	–	–	–	–
3. Carbohydrates and/or glycosides	–	–	–	+	+	+
4. Flavonoides	–	±	+	+	+	+
5. Tanins	–	–	–	+	+	+
6. Saponins	–	–	–	–	–	–
7. Anthraquinones	–	–	–	–	–	–
8. Alkaloides and/or nitrogenous bases	–	–	–	–	–	–
9. Protein and/or amino acids	–	–	–	–	+	+

(–) absent (b) brown (d.b.) dark brown (g) green (+) present (s.s) semi-solid (s) solid.

Table 2Percentage yield of successive extracts analysis of *Euphorbia cuneata* Vahl.

Extracts	Pet. ether	Ether	Chloroform	Ethyl acetate	Butanol	Alcohol 95%
Percentage	5.23 ± 0.29	1.66 ± 0.31	2.89 ± 0.11	4.80 ± 1.19	7.20 ± 1.29	10.7 ± 1.01

Table 3Total percent of primary and secondary metabolites of *Euphorbia cuneata* Vahl.

Phytochemical contents	Primary metabolites			Secondary metabolites		
	Carbohydrate	lipids	Protein	Flavonoids	Phenolic	Tannins
Percentage	6.25 ± 1.11	5.12 ± 1.40	7.15 ± 1.31	11.26 ± 1.02	9.15 ± 1.21	5.23 ± 1.29

Table 4Quantitative phytochemical analysis of *Euphorbia cuneata* Vahl.

Parameter	Moisture	Total ash	Acid insoluble ash	Water soluble ash
Percentage	4.87 ± 2.09	7.34 ± 2.11	2.15 ± 1.27	6.28 ± 1.25

Table 5Estimation of total, protein and free amino acids percentage in of *Euphorbia cuneata* Vahl.

No.	R _t	Amino acids %			
		Protein hydrolysate	Total	Protein	Free
1	11.53	Aspartic	7.64	5.32	2.32
2	14.85	Threonine	6.93	4.18	2.75
3	16.26	Serine	8.59	6.59	4.08
4	18.47	Glutamic acid	6.81	4.51	2.3
5	25.54	Glycine	5.15	3.63	1.15
6	26.87	Alanine	6.92	4.47	2.45
7	30.22	Valine	6.38	4.71	1.67
8	32.57	Methionine	4.48	3.34	1.14
9	34.20	Isoleucine	5.25	3.32	1.93
10	35.44	Lucien	5.85	3.04	2.81
11	39.70	Tyrosine	3.52	2.72	0.80
12	42.40	Phenyl alanine	4.11	2.26	1.85
13	50.57	Histidine	4.39	2.37	2.02
14	54.19	Lysine	4.28	3.01	1.27
15	63.02	Arginine	20.86	15.58	5.28

percentage of secondary metabolites indicate that the plant has potential to act as a source of useful drugs and act as good medicinal plant (Akinyeye et al., 2014).

The Pharmacopoeia Constants determination (Table 4) Showed that the moisture contents of the plant was not very high (4.87 ± 2.09) and this is accepted with the desert condition which the plant grow in while water soluble ash (6.28 ± 1.2) which mean that the active principles in the plant are high.

Upon analysis of amino acids contents in the Aerial part of *Euphorbia cuneata* Vahl. 15 amino acids were detected with different percentage of amino acids contents in deferent types (Total, free and protein hydrolysate). Arginine represented the highest concentration (20.86).

4. Conclusion

Euphorbia cuneata Vahl one of the **best** genus of family Euphorbiaceae because it's of great importance as medicinal plant which attributed to its contents of various phytochemical constituents with high concentration. It also can be of great important for animal and human in time of famine due to its high protein contents.

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