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FORMULATION AND EVALUATION OF *GREWIATENAX* FRUITS AS EFFERVESCENT TABLETS FOR TREATMENT OF IRON DEFICIENCY ANEMIA

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ABSTRACT

Grewiatenax fruits as nutritional supplement are being widely used in traditional medicine in most parts of Sudan to treat anemic patients. This study was conducted to specify the most productive method for extracting*G. tenax* fruits and to standardize and formulate the extract into effervescent tablets. The fruits were extracted by hot and cold extraction with maceration using 80%, 65%, and 50% ethanol strength and the extracts were standardized. Two formulae (F1 and F2) were prepared from the extract and compressed into tablets. The formulated tablets were subjected for quality control tests. The 80% ethanol extract showed the highest extract yield and iron contents comparing to other extracts. The formulated tablets of both formulae showed good quality and pass all quality control tests.*G. tenax* fruitsextract could be pharmaceutically formulated into tablets which exhibited good quality control.To maximize yield value of*G. tenax* fruitsextract, maceration method using 80% ethanol strength is of paramount importance.

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INTRODUCTION

Plants remain the main source of medicines for a large proportion of the world's population, particularly in the developing world, despite the advent of the pharmaceutical chemistry during the early twentieth century (Ahmad*et al.,* 2006). Aboagarib*et al.*, (2014)reported that *Grewiatenax* (*G. tenax*) is one of the valuable plant species in Sudan and it is widely spread in arid area such as soil sand and near mountains, especially in the Savanna plantation area of the Northern and Middle of Sudan. *G.tenax*belongs to the family Tiliaceae which distributed throughout the western and eastern Sahelian zones, northern and southern Africa, the Arabian Peninsula and is also reported to grow from Iran to India on a wide array of soils, the juice made from its fruit is used as refreshing drink during the hot summer season (Gebauer*et al.,* 2007).

It has been reported by Muhammad (2009)that due to its high iron content, fruits of *G. tenax*are often used in special diets for pregnant women and anemic children and its leaves and twigs are palatable fodder for livestock and also important components of folk medicine for the treatment of trachoma, tonsillitis, infections and are used as a poultice to treat swelling. Gebauer*et al*. (2007) stated thatthe dry fruit pulp contains 6.3% crude protein, 0.4% fat, 8.1% fiber, 4.5% ash, and 15.1% starch and the iron content has attracted most attention which generally reaches 7.4 mg /100 gram, the fruits also contain many other minerals such as sulfur (0.1%), phosphorus (0.08%), magnesium (0.17%), calcium(0.61%), sodium (0.01%), and potassium (1.45%), the pulp sweetness is provided by D-fructose (24.3%), glucose (21%), and sucrose (1.6%).It was documented that *G. tenax* fruit contained higher amounts of crude protein, crude fiber and carbohydrates and its nutritional value lied in its good content of iron, ascorbic acid, D-fructose sugar and calcium (Sulimanet *al.,* 2018). It has

been reported that in traditional medicine leaves, root, and fruits of *G. tenax* are used for the treatment of digestive diseases, liver disorders, jaundice, and inflammatory conditions (Safa *et al.,* 2012). Furthermore, it was stated that the administration of ethanol extract of *G. tenax* significantly restored CCl_4 induced biochemical and histopathological changes and significantly reduced cholesterol, low-density lipoproteins, and triglycerides level (Safa *et al.,* 2012) and attributed these effects to antioxidant and anti-inflammatory properties. Experimental studies in rats showed no adverse effect of ethanolic extract of *G. tenx*except mild diarrhea in the high dose of 2 g/kg b.w.(Al-Asmari*et al*., 2014). Alzergy (2017) suggested that administration of *G. tenax* fruit for two weeks in traditional treatment inhibited oxidative effects resulted from formalin exposure and attributed that to flavonoids and other antioxidant constituents in this plant.
However, it has been reported that all parts of *G.tenax* are a rich source of medicinally useful components; the leaves are easily available in abundant amounts and have been intensively used in traditional medicine for the treatment of venous insufficiency, hemorrhoids, hypoglycemia, diarrhea and fungal or microbial infectionsSyeda (2014). The methanolic extract of *G. tenax* stem bark showed antibacterial activity against Bacillus sublilis bacteria (Ali*et al*., 2017).

On contrast, Ayed *et al.* (2015) concluded that enterohepatonephropathy is a characteristic feature of *G.tenax* toxicities in chicks and these lesions were correlated with changes in serum aspartate aminotransferase activity and concentrations of total protein, albumin, globulin, total bilirubin, cholesterol, uric acid, and calcium as well as with alterations in hemoglobin, packed cell volume, red blood cells, mean corpuscular volume and mean corpuscular hemoglobin concentration values. Effervescent tablets are uncoated tablets generally containing acid substances and carbonates or hydrogen carbonates, which react rapidly in the presence of water to release carbon dioxide (effervescence). They are intended to be dissolved or dispersed in water before administration (USP, 2009) and are used to obtain rapid drug action, or to facilitate the intake of the drug(Aulton, 2002). Beside that effervescent tablets can enhance the palatability of the drugs (James, 2007). On the other hand effervescent dosage forms are relatively expensive to produce and contain a high Na and/or K concentrations which can affect some patients populations under Na or K restriction (James, 2007). Therefore, this study was intendedto specify the best method of extraction through the yield value percentages and the elemental analyses for iron determination and to evaluate tablets of the formulae formed by the study.

MATERIALS AND METHODS

Collection of *G. tenax* fruits: First class freshly handpicked *G. tenax*fruits were purchased from Omdurman City market, Khartoum, Sudan. The *G. tenax*plant isgenerally grown inSouth Darfur, West of Sudan. They fruits were clean with bright reddish yellow color. Fruits were authenticated in Medicinal and Aromatic Plants Research Institute. Extraction and chemical analyses

Extraction of *G. tenax* fruits

Cold extraction method: Maceration was done according to Saeed and Elmubarak(1974), where 100 gm of clean and freshly collected *Grewiatenax* fruits macerated in 400 ml of ethanol of different strengths (50%, 65%, and 80%), after removing solvents, the dried fruits extract was collected and the yield value of each concentration was calculated.

Hot extraction methods

Digestion method: Hot extraction was done according to Soxhelt (1879), where100gm of fruits was macerated in the same strengths of 80%, 65%.and 50% ethanol.

Decoction method: A total of 100 gm of *G. tenax* fruit was washed with distilled water for 30 seconds and transferred into a 1 L glass flask, 450ml of distilled water was added and the flask content was heated at 100 ºC for twenty minutes, then removed from the heater and the temperature allowed to fall down till reach 55ºC, the fruits were squeezed thoroughly and gently by hands and transferred into a glass beaker which contain 1.5 gm of sodium benzoate dissolved in 5ml distilled water to prevent fermentation so that fermentation can be prevented, flask content was gently shaken for three minutes before filtration througha cotton pad, concentrated with a rotary evaporator and poured into Petri-dishes to drying at room temperature.

Standardization of *G. tenax* extracts

Physical characteristic of *G. tenax* extract: Extracts of *G. tenax* were subjected to sensory attributes (color, odor, consistency, taste and solubility in water) using hedonic scale. The results obtained by the panelists**.**

Yield value calculation of extraction methods: The yield value was calculated after the extract has been dried.

Ironanalysis: Iron analysis for extracts of *G. tenax* were prepared according to James (2007)**,** where two parts by weight of Nitric acid and one part of perchloric acid were mixed with the crude fruits**.** 0.5g of each air-dried *G. tenax*fruits extract was accurately weighed, finely cut and homogeneously mixed in a clean silica crucible. 10ml of digestion mixture was added and placed in an oven, slowly heated to 100°C and maintained at this temperature for up to 3 hours, then heated to 120°C for 2 hours. The temperature rose very slowly to 240° C, avoiding loses due to possible violent reactions especially in the temperature range of 160−200°C, and maintained at this temperature for 4 hours, the remaining of dry inorganic residue dissolved in 10ml of nitric acid and transferred into 100ml volumetric flask and the volume completed with water. The samples were prepared in duplicate and the mean was calculated.

Isolation of *G. tenax* fruits extract: Isolation of *G. tenax* fruits extract done by using preparative thin layer chromatography (TLC), dissolved in a solvent and allowed to dry at room temperature, scanning was done using Ultra-Violet (UV) and Infra-Red (IR).

Tablets preparation of *G. tenax* extracts: The already prepared extract of 85% ethanol was chosen to prepare the two types of tablets. Ingredients, which shown in Table 1, wereaccurately weighed and transferred to stainless steel pan. Citric acid, tartaric acid, methyl crystalline cellulose (MCC) and lactose was added to the extract, and thoroughly mixed with pestle for 45 minutes till the ingredients were homogeneously mixed, the wet mass was then forced manually through No.10 mesh screen. The formed granules were

collected in aluminum plates and dried in a hot air oven at 55ºC for 48 hour. On the other hand, the sodium bicarbonate was poured into other stainless pan and wetted with methanol, the wet mass was then forced through No.10 mesh screen and the formed granules were collected on aluminum plates and dried in a hot air oven at 55ºC for 48 hours. All the dried granules were resized by forcing them through No. 32 mesh screen. Finally, (51 gm) of MCC was added and mixed thoroughly for 20 minutes. Orange color and flavor were dissolved in 35ml of methanol, filtered, packed into glass sprayer and sprayed on the granules and the sodium benzoate separately, with continuous mixing of the granules. The granules were then transferred to the oven at 75 ºC for 25 minutes with mixing after each 5 minutes. The sodium benzoate was triturated with mortar and pestle until a homogeneous color and small particle size were obtained and thoroughly mixed with the granules bed.Preformulation studies were done for granules (particle size and distribution, powder flow ability and compressibility). The amount of MCC in F1 and F2 formulae were divided into 300 and 200gm in the granulation step and 51 and 49 gm in the pre-compression step respectively. The bicarbonate was wetted with 10 ml and 5ml methanol in formula A and B respectively.The formulation ingredients per tablet prepared by this study of formulae F1 and F2 were shown in table 1. The granule mixture was compressed into tablets using single punch tableting machine and lubricated with a small amount of liquid paraffin to prevent adhesion and chipping of tablet and promote smooth ejection, 20 mm die size was mounted and the compression force and lower punch level were adjusted, the in-process control was carried out to ensure constant weight of tablets, hardness and effervescence time. The formed tablets were collected and packed in well closed glass container.

Table 1. The formulation ingredients per tablet of formulae F1 and F2 prepared by this study

Materials	F1	F2
	Quant./tab(mg)	Quant./tab(mg)
Extract	511.00	511.00
MCC powder	585.00	415.00
Lactose powder	165.00	415.00
Sodium benzoate	80.00	0.00
Citric acid (anhydrous)	95.20	95.20
Tartaric acid	190.30	190.30
Sodium bicarbonate	323.50	323.50
Orange color & flavor	50.00	50.00

Quality control tests for formed tablets: Weight variation test, hardness test, friability test and disintegration time test for both formula were carried out, where six tablets were randomly taken from each formula, each tablet was placed in glass beaker containing 200ml distilled water at 28º C. the time required to complete the effervescence reaction was calculated, and the average time was compared to the British Pharmacopoeia specifications (2009). Content uniformity test was also done, where ten tablets from both formulae were individually dissolved in 200ml distilled water at 28º C and analyzed using the UV spectrophotometer according to USP[17], for calculation of content uniformity calibration curve was prepared (Figure 1).

RESULTS AND DISCUSSION

Standardization of *G. tenax* fruits extracts

Physical Characteristics: Physical Characters of the extracts were shown in table 2. **Color:** All colors of maceration and digestion extracts using ethanol 80% were deep yellow and the color was pale yellow of extracts using ethanol 65% while the color was tend to be pale brown of extracts using ethanol 50%.

Odor: Odor was distinct in all extracts in different extraction methods.

Consistency: All of maceration extracts were moderately viscous, digestion extract of 80% ethanol strength was liquid and semisolid in both 65% and 50% ethanol strength digestion extracts, decoction extract was very viscous (plastic) while seemed to be sticky in extract using extraction method of maceration followed by freeze drying.

Taste: taste was sweet in all extracts using the different types of extraction methods.

Solubility in water: All extracts were soluble in water except extract of maceration followed by freezing, which was poorly soluble in water, while extract of decoction method was sparingly soluble in water (Table2).

Calibration Curve (UV)

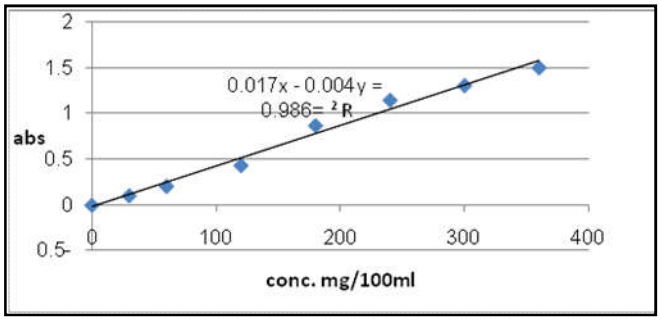

Figure 1. The UV calibration curve of the 80% ethanol maceration extract

Yield value of extraction methods: Yield value of extraction methods of the Extracts were shown in table 3, the highest yield of extract was that of using maceration method of 80% methanol strength (35.70) seconded by digestion method (30.35) and then followed by decoction method (28.80), while the method of maceration followed by freeze drying showed the lowest extract yield value (22.60). It could be noticed that, in maceration extraction method, the yield value decreased by decreasing methanol strength. The highest yield value and the highest iron content of the extracts was obtained by maceration using ethanol 80%; make this extract of best choice for formulation into pharmaceutical dosage form (effervescence tablet). On the other hand, Freeze drying and decoction methods produce no result in mineral analysis, this may be attributed to the formation of gel structure owning from higher temperature used in decoction method while the lower temperature in freeze drying may express the plastic consistency of both of the extracts obtained by these methods, in addition, adding sodium benzoate (to compact the microbial attack) to decoction method may alter chemical composition of the extract. Therefore, the selection of water as extraction solvent should be excluded. Digestion method of extraction (using ethanol 80%) produces stable concentrated liquid which retains its initial characteristics for more than one year without any change in its appearance and consistency; therefore, it can be effectively and easily formulated as syrup dosage form as

effective nutraceutical dosage form for children and elder patients suffer from iron deficiency anemia.

These values can be comparable to the result obtained by this study when using maceration extract by 65% methanol

Table 2 The physical characteristics of the extracts

Extraction method	Ethanol strength	Color	Odor	Consistency	Taste	Solubility in water
Maceration	80%	Deep yellow	Distinct	Moderately viscous	Sweet	soluble
	65%	Pale yellow	Distinct	Moderately viscous	Sweet	soluble
	50%	Pale brown	Distinct	Moderately viscous	Sweet	Soluble
Digestion	80%	Deep yellow	Distinct	Liquid	Sweet	soluble
	65%	Pale yellow	Distinct	Semisolid	Sweet	soluble
	50%	Pale brown	Distinct	Semisolid	Sweet	Soluble
Decoction	-	Pale brown	Distinct	Very viscous (plastic)	-	Sparingly soluble
Maceration followed by freeze drying	-	Pale yellowish-red	Distinct	Sticky	sweet	Poorly soluble

Table 3. The yield value for the extracts

Extraction method	Alcohol percent	Yield value % ±2
Maceration	80%	35.70
	65%	27.81
	50%	13.67
Digestion	80%	30.35
	65%	27.32
	50%	31.52
Decoction	-	28.80
Maceration followed by freeze drying	-	22.60

Table 4. Iron content of the extracts

Sample name	Iron conc. /0.5 gram Analysis 1	Iron conc. /0.5 gram Analysis 2	Mean Iron conc. /0.5 gram
Maceration80% ethanol	35.20 µg	33.36 µg	34.28 µg
Maceration 65% ethanol	20.37 µg	22.27 µg	21.32 µg
Maceration 50% ethanol	18.16 µg	20.18 µg	19.17 µg
Digestion 80% ethanol	14.17 µg	12.05 µg	13.11 µg
Digestion 65% ethanol	05.78 µg	07.31 µg	06.55 µg
Digestion 50% ethanol	05.10 µg	03.95 µg	04.53 µg

Figure 2. TLC chromatogram

Iron content: Iron content of extracts was shown in Table 4; the highest iron content was that of using maceration method of 80% methanol strength; mean value (34.28 µg/0.5g), seconded by 65% methanol strength; mean value (21.32 µg/0.5g) and then followed by 50% methanol strength; mean value (19.17 µg/0.5g), while the digestion method showed iron values of 13.11 µg/0.5g, 06.55 µg/0.5g and 04.53 µg/0.5g using 80%, 65% and 50% ethanol extract, respectively. Ali *et al.* (2016) reported that *G. tenax* fruits contain 21-30mg Fe/100g whereas Abdualrahman*et al.*(2011) stated that *G. tenax* fruits were found to contain 25 mg/100g iron. Mohammed Elhassan and Yagi(2010) investigated *G. tenax* fruits components and found that potassium showed higher content (817mg/100g) than calcium (790mg/100g) while iron was found to be 21mg/100g.

strength (mean value: 21.32 µg/0.5g), and lower than the result obtained by this study when using maceration extract by 80% methanol strength (mean value: 34.28 µg/0.5g). These results support the use of *G. tenax* in various region of the world with regard of the traditional treatment of anemia. The iron content present in *G. tenax* fruits not high enough to compensate the iron needs for patients suffer from iron deficiency anemia. Small amounts of iron in the presence of ascorbic acid (vitamin C) and the other phytochemical groups may be responsible for iron absorption from the extract, in addition, the presence of ascorbic acid in *G. tenax* fruits[5] may act as absorption factor for dietary iron; this explains the increase in packed cell volume and elevation in haemoglobin concentration after taking *G. tenax* fruits.

Figure 3. The UV scanning for the extract showed maximum absorbance at 280 nm

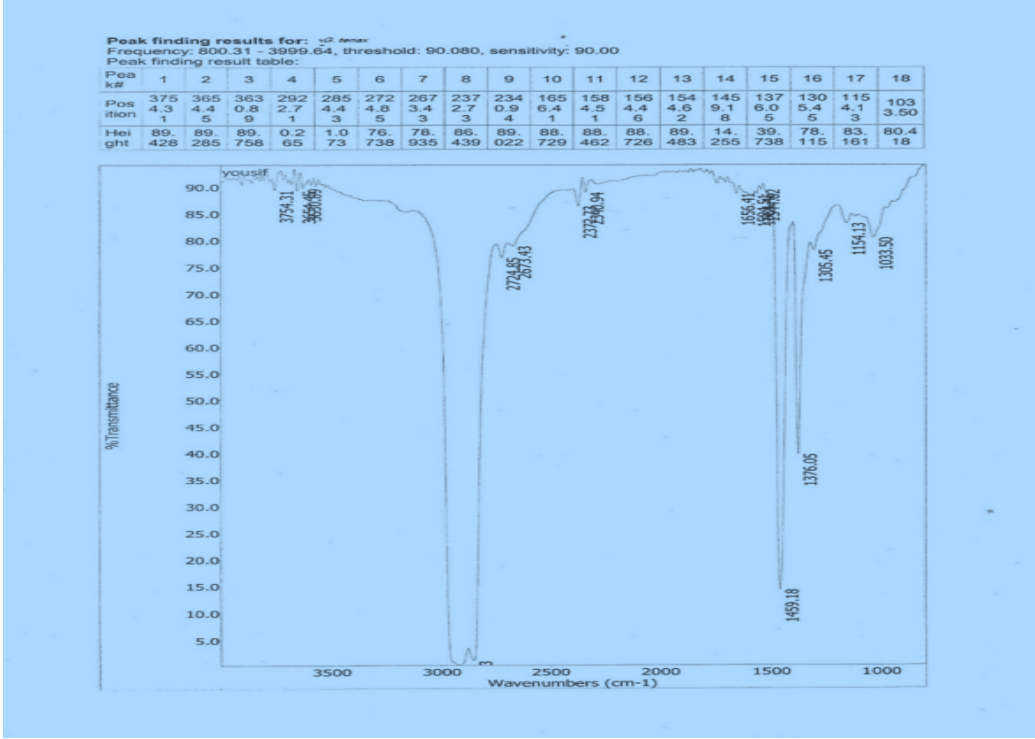

Figure 4. The IR spectrum for the *G. tenax*extract

Table 4. The Angle of ribose, Carr's index for both F1 and F2 granules blend

Parameter	F1	F2
Angle of ribose	19 º	27 º
Bulk Density (g cm^{-3})	0.544	0.614
Tapped Density (g cm^{-3})	0.636	0.766
Carr's Index %	14.465	19.843

Granules flowability: Angle of ribose of granules showed that both of the prepared formulae were good, F1 was 19 º while F2 was 27 º. Bulk Density, Tapped Density and carr's Index % showed compliance to the recommended range of compressibility (Table 4).

Particle Size Analysis and Distribution: Particle size analysis of granules showed that both of the prepared formulae were in range, no remarkable differences noticed between the two formulae with regard to particle size and distribution (Table 5).

Quality control tests of tablets: Weight variation, hardness, friability, content uniformity and disintegration tests for both formulae were within the permitted limits from at zero time of tablet production up to three months after production at accelerated condition of temperature (40°C and 75% RH), this reflects the stability of both formulae (Table 6). Both formulae pass weight variation test, percent deviations for both formulae were within the limit (less than 5%), Hardness test for both formulae were within the limits (more than 4kg and less than 10kg), Friability test results for both formulae were within the limit (less than 1%), Content uniformity test for both formulae

Table 5: The particle size distribution of final- blended granules of both formulae

Mesh No.	Aperture Size Range / Mean (μm)	F1		F2	
		Weight (gm) or Frequency	*Percent Frequency (%)*	*Weight(gm) or Frequency*	*Percent Frequency (%)*
24	600- 850 μm, 710 μm	0.60	6.38	0.40	04.30
32	425 -600 μm, 500 μm	1.80	19.15	2.10	22.58
42	300- 425 μm, 355 μm	3.30	35.11	2.30	24.73
60	212 -300 μm, 250 μm	2.50	26.60	3.10	33.33
80	150 -212 μm, 180 μm	0.70	7.45	0.60	6.45
100	125 -180 μm, 150 μm	0.50	5.32	0.80	8.60

Table 6. Mean variation of tablets quality control tests

Weight variation wt.(g)		Friability test %weight loss)(		Hardness kg)(		Content Uniformity Content%)(		Disintegration time (min.)	
F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
2.038	2.032	0.792	0.812	7.131	6.092	102.488	103.347	2.34	2.67

F1 and F2: Formulated tablets under investigation

was within the limits (not less than 85% and not more than 115%), and effervescence time test (disintegration test) was also within the specified time (less than 5 minutes). The effervescence time test can be considered as disintegration test and dissolution test because, the tablet was completely soluble within the specified time for effervescence.

Conclusion

The 80% ethanol extract showed the highest extract yield and iron contents comparing to other extracts, to maximize yield value of*G. tenax* fruitsextract, maceration method using 80% ethanol strength is of paramount importance. The formulated tablets of both formulae showed good quality and pass all quality control tests. *G. tenax* fruitsextract could be pharmaceutically formulated into tablets which exhibited good quality control.

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