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Standardisation and physicochemical characterisation of the extracts of seeds of *Glinus lotoides*

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Extraction methods were standardised for saponin-containing extracts from the seeds of *Glinus lotoides* and the effects of some extraction process variables, such as the extracting solvent (various concentrations of methanol in water) and method of extract drying (freeze-drying and vacuum oven-drying), on the physical properties of the extracts were investigated. Physicochemical properties, namely particle size and size distribution, morphology, water uptake profiles and sorption isotherms, densities, flow properties and compaction profiles, of the crude dry extracts of 60% methanol (extract A), 70% methanol (extract B) and 80% methanol (extract C) were investigated. The average particle sizes (X_{50}) of extracts A, B and C were found to be 68.4, 92.1 and 68.5 μm, respectively. Scanning electron micrographs of freeze-dried and vacuum oven-dried extract A showed that the particles are irregular in shape and are compact masses with sharp edges. The percent water uptake by the crude extracts was found to increase with an increase in relative humidities, while the hygroscopicity increased with decreasing methanol ratio of the extracting solvent. The bulk and the true densities of the three extracts (A, B and C) ranged from 0.66 to 0.67 and 1.49 to 1.50 g/ml, respectively. The tapped density (0.94 g/ml) and hence the porosity (56.0%), Carr's index (29.8%) and Hausner ratio (1.42) of extract A were greater than those of extracts B and C. Measurements of angle of repose indicated that all of the extracts exhibit poor flow properties. Compaction studies revealed that extract C has higher compactibility than extracts A and B.

1. Introduction

Glinus lotoides Linne (Family: Molluginocea), locally known as "Mettere" is a traditional taenicidal herb in Ethiopia (Pankhurst 1965). The cestocidal activity of *G. lotoides* has been attributed to saponins it contains (Abegaz and Tecle 1980). A description of the plant, the traditional mode of administration, methods of extraction and its *in vitro* and *in vivo* taenicidal activity against *Tenia saginata* and *Hymenolepis nana* worms have been reported elsewhere (Kloos et al. 1978; Djote 1978; Endale et al. 1997, 1998).

Previous work on extraction from the seeds of *G. lotoides* has utilised 80% methanol in water (Djote 1978; Endale et al. 1997, 1998). However, there is no evidence of any attempt to show the suitability of this solvent system over others with respect to its saponin yield. It has been reported that alterations in the extraction process such as, solvent ratio, rate of mixing, time of extraction, etc., may alter both the quantitative ratio and the qualitative composition of the extract (List and Schmidt 1989). Hence, in this study, the saponin extracting powers of aqueous methanol solvents of various proportions from the seeds of *G. lotoides* have been investigated to determine which solvent system gives maximum yield.

Formulation of plant extracts is a challenge in pharmaceutical technology due to the complex physicochemical properties of the extracts (Luftensteiner and Viernstein 1999). Physicochemical properties, such as particle size, surface area and surface volume ratio of a particle, moisture content and densities, can be related to the physicochemical and pharmacological properties of a drug. In the manufacture of solid dosage forms, the particle size and size distribution of the drug and excipients can influence a number of important factors, such as flow properties. This applies particularly to the formulation of tablets using direct compression. Moisture modifies the flow and mechanical properties of many powders particularly dry plant extracts. With most hygroscopic materials, changes in moisture level can greatly influence many important parameters, such as chemical stability and compatibility (Wells 1988). Hence, investigation of some physicochemical properties of the dry extract is imperative before commencing formulation.

The present study, therefore, reports on the standardisation of the extraction process (extracting solvents and methods of extract drying), and the physicochemical characterisation and compaction profiles of the dry extracts.

2. Investigations, results and discussion

2.1. Standardisation of the extraction method

The presence of fats in the starting materials not only interferes with the extraction and drying process but also with formulation. Thus, removal of fats from plant materials, particularly from seeds and other organs with a high fat content, is advisable prior to the main extraction step.

The amount of fat removed from the seeds of *G. lotoides* at three successive defatting steps was found to be 62.5%, 23.6% and 9.7%, respectively. The total fat content of the seeds was found to be 14.3%, which is in good agreement with a previous report of 14% oil (Biftu et al. 1979). The three successive defatting procedures removed a total of 95.8% of the total fat indicating that all three defatting steps are required to remove most of the fat.

Total extraction of saponins from plant materials is usually difficult. This challenge is generally associated with their relatively large molecular weight and high polarity (Hostettmann and Marston 1995). Knowing the total amount of saponin in the plant material is, however, important for the development of an analytical method as well as to select a suitable solvent system for the maximum yield of saponins.

Exhaustive extraction of the seeds (10 successive extractions with 80% methanol) yielded 18% crude extract, 6.8% n-butanol fraction and 3.6% purified extract (total saponins) calculated on the dry defatted seeds. This result, shows that the extraction method described earlier (3 successive extractions with 80% methanol) (Djote 1978; Endale et al. 1997, 1998) removes less than 60% of the total saponins, thus indicating the challenge posed by the total extraction of saponins from the seeds of the plant.

Table 1 shows the amount of the various extracts obtained using solvent systems with different proportions (0–100% methanol in water). The amounts of crude extract, n-butanol fraction and purified extract were determined as described elsewhere (Endale 2000). As shown in the Table, the amount of crude extract obtained increases with increasing proportion of water, the maximum being 16.5% with pure water. However, the n-butanol fraction and the purified extract showed maximum yields with 20% and 60% methanol, respectively.

Based on the relative amounts of saponins in the crude extracts and the convenience of steps in the extraction process, such as filtration, solvent systems namely, 60%, 70% and 80% methanol were selected and the physicochemical properties of the crude extracts (A, B and C) obtained with them were characterised.

Table: Yield in percent of the crude extract, n-butanol fraction and purified extract from 5 g of *G. lotoides* seeds using various concentrations of methanol in water as extracting solvent

Methanol in water (%)	Crude extract g (%)	n-Butanol fraction g (%)	Purified extract g (%)
0	0.824 (16.5)	0.222 (4.44)	0.097 (1.94)
10	0.801 (16.0)	0.243 (4.85)	0.101 (2.01)
20	0.775 (15.5)	0.268 (5.36)	0.114 (2.28)
30	0.765 (15.3)	0.265 (5.30)	0.125 (2.49)
40	0.750 (15.0)	0.232 (4.64)	0.132 (2.63)
50	0.702 (14.0)	0.220 (4.40)	0.148 (2.95)
60	0.670 (13.4)	0.211 (4.22)	0.155 (3.10)
70	0.605 (12.1)	0.210 (4.20)	0.130 (2.60)
80	0.560 (11.2)	0.160 (3.20)	0.120 (2.40)
90	0.455 (9.1)	0.190 (3.80)	0.115 (2.30)
100	0.440 (8.8)	0.266 (5.32)	0.087 (1.73)

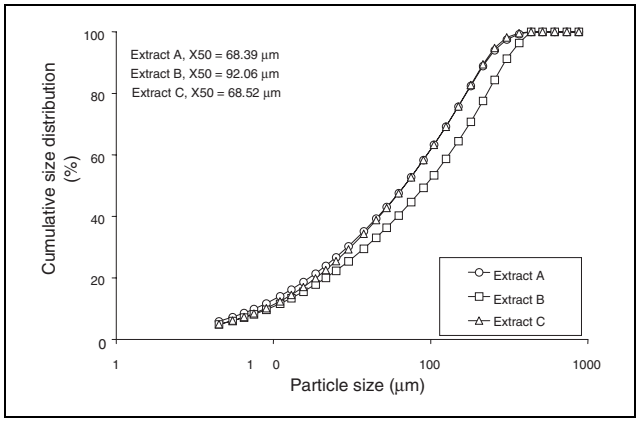

Fig. 1: Cumulative particle size and size distribution of extracts A, B and C of the seeds of *G. lotoides*

2.2. Characterisation of the dry crude extracts

The particle size and size distributions of extracts A, B and C were studied using a Sympatec laser diffraction spectrometer. Fig. 1 shows the particle size and size distribution of the various crude extracts. As shown in the Fig., the average particle sizes (X_{50}) extracts A, B and C were found to be 68.39, 92.06 and 68.52 µm, respectively. Extracts A and C showed similar particle size distributions, (in fact the curves are superimposed), whereas extract B showed relatively larger particles.

Fig. 2 shows a scanning electron micrograph of vacuum oven dried crude extract of the seeds of *G. lotoides* at a magnification of 2000. As shown in the micrograph, the particles of the vacuum oven dried crude extract are irregular both in shape and in size and are relatively compact masses with sharp edges. On the other hand, the micrograph of the freeze-dried crude extract, (Fig. 3) shows it to be composed of aggregated particles of irregular shape. The purified extract, however, appears spherical, relatively regular and composed of very small particles (Fig. 4). Thus, the irregularity of shape of the crude extracts may be attributed to the presence of extraneous materials.

Many drug substances, particularly plant extracts, have a tendency to adsorb moisture. Fig. 5 shows the water uptake profiles of freeze-dried crude extract (extract C) at different relative humidities at 20 °C. The percent water uptake by the crude extract increases with an increase in relative humidity. Moreover, at higher relative humidity, the powdered extract requires a longer time to reach maximum and equilibrium water uptake.

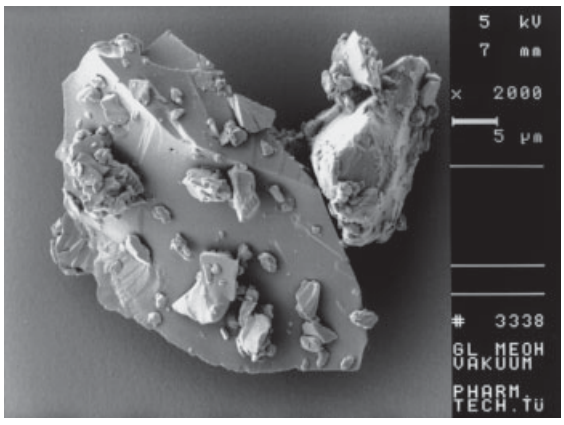

Fig. 2: Scanning electron micrographs of the vacuum oven-dried extract A of the seeds of *G. lotoides* (2000 X)

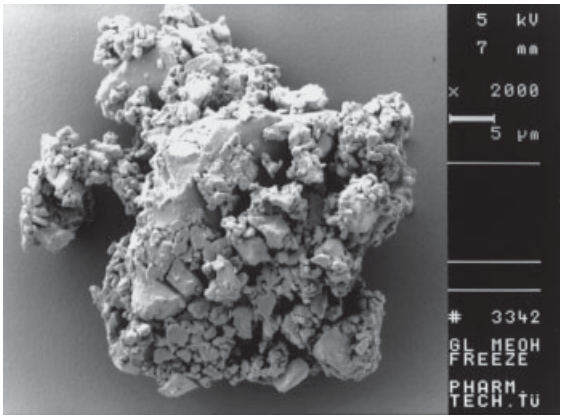

Fig. 3: Scanning electron micrographs of the freeze-dried extract A of the seeds of *G. lotoides* (2000 X)

Water sorption isotherms of the various extracts were determined from the equilibrium water uptakes of the extracts at different relative humidities. Such representations have the advantage of indicating the water uptake profile of the various extracts at different relative humidities. Based on sorption isotherm results, the necessary precautions could be taken during manufacturing and storage.

Fig. 6 depicts water sorption isotherms of freeze-dried and vacuum oven-dried extract A, the n-butanol fraction and the purified extract of the seeds at 20 °C. The Fig. also demonstrates the significant reduction in hygroscopicity of the extracts following purification. The water uptake of the extract was reduced from 40.5% to 14.0% and 10.8% at 85% RH as a result of purification of the freeze dried crude extract to the n-butanol fraction and purified extract, respectively. Furthermore, the sorption isotherms of the crude extract show a marked increase in water uptake only above 60% relative humidity, indicating that the hygroscopicity of the crude extracts is manageable at lower relative humidities.

The physical properties of the dry extracts depend, in addition to other factors, on the drying process. Fig. 6 also shows the effect of the drying process on the water sorption isotherms of crude extracts. The freeze-dried crude extracts are more hygroscopic than vacuum oven-dried extracts, particularly at higher relative humidities. At 85% RH, the percent water uptakes of the crude extracts were found to be 40.5% and 22.0% (Fig. 6) for freeze-dried and vacuum oven dried, respectively. The higher water sorption properties of freeze-dried extracts may be attributed to the higher surface area of these products, as mentioned elsewhere (Mishra et al. 1996; Costantino et al. 1997).

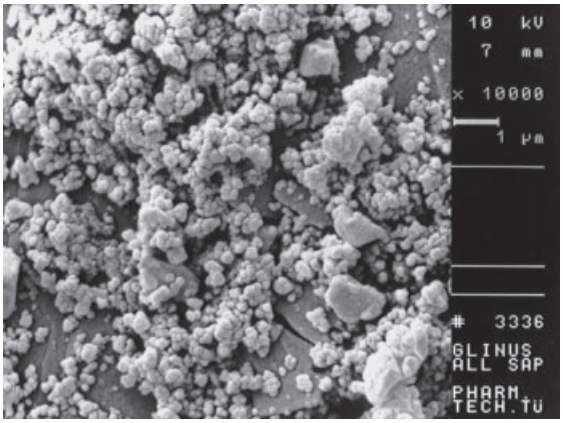

Fig. 4: Scanning electron micrographs of the purified extract of the seeds of *G. lotoides* (10,000 X)

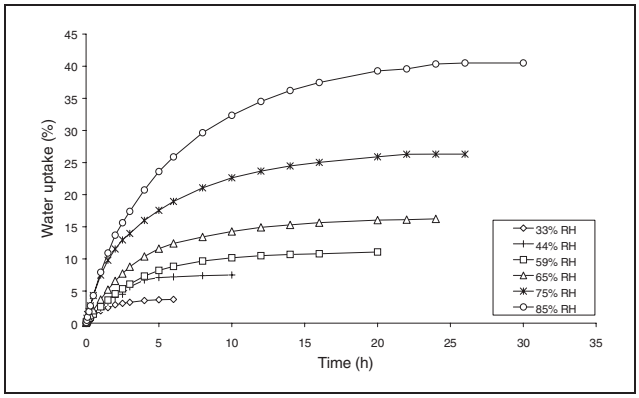

Fig. 5: Water uptake profiles of the freeze-dried extract A of the seeds of *G. lotoides* at different relative humidities at 20 °C

Water sorption isotherms of extracts A, B and C indicate that the relative hygroscopicity of the extracts differs mainly at the higher relative humidities, 75% and 85%. Moreover, the hygroscopicity of the extracts increases with a reduction in the proportion of methanol in the extracting solvent. Hence, extract A is more hygroscopic than extracts B and C. This could be attributed to the presence of more water-soluble compounds in extract A (60% methanol) than in those containing a lower percentage of water, extracts B and C (70% and 80% methanol, respectively). Karl Fischer titration of the extracts confirmed the variation in water content. Thus, extracts A, B and C contained 3.63% (S.D. 0.11), 3.5% (S.D. 0.13) and 3.36% (S.D. 0.14) water, respectively.

Density is an important physical characteristic of pharmaceutical powders. Uniformity of dosage, powder flow property and compressibility can be affected by density. Extracts A, B and C show similar bulk and true densities, in the range 0.66–0.67 and 1.49–1.51 g/ml, respectively. However, extract A showed a higher tapped density (0.94 g/ml) than extracts B (0.85 g/ml) and C (0.84 g/ml).

Porosity, consolidation index (Carr's index) and Hausner ratio, which are measures of flowability were calculated (Wells 1988; Fonner et al. 1966; Wells and Aulton 1988) from the density results of the extracts. Extract A exhibited a higher Carr's index and porosity (29.8, 56%) than extracts B (21.2, 55.4%) and C (20.2, 55.5%). Thus, the flow of extract A is considered poor and those of extracts B and C fair or passable (Wells and Aulton 1988).

Consolidation, a term used interchangeably with compaction, describes the compression profiles of powders. Carr's index, however, does not always reflect the ease or speed

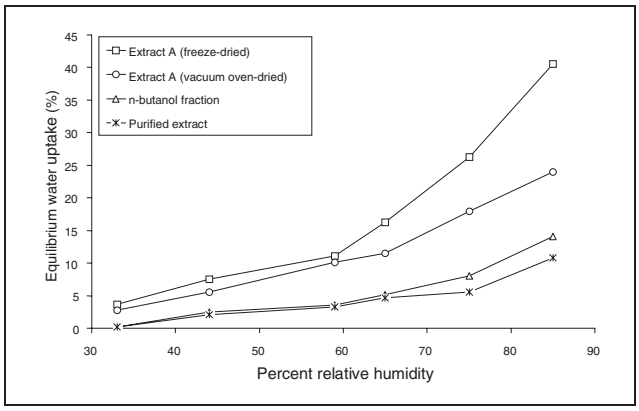

Fig. 6: Water sorption isotherms of the freeze-dried and vacuum oven-dried extract A, n-butanol fraction and purified extract of the seeds of *G. lotoides* at 20 °C

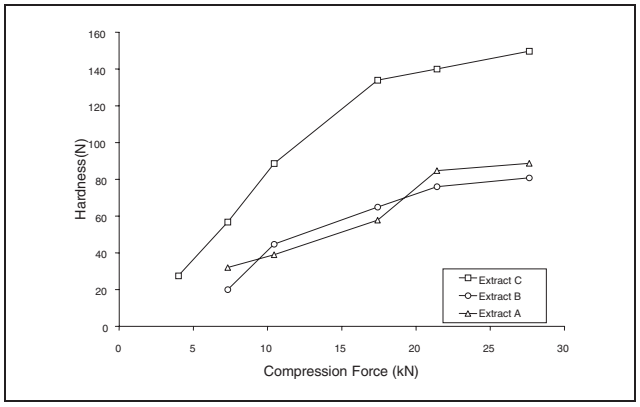

Fig. 7: Compaction profiles of extracts A, B and C of the seeds of *G. lotoides*

with which consolidation of powder occurs, as it is a one-point determination. Indeed, some materials having a high index (suggesting poor flow) may yet consolidate rapidly (Wells and Aulton 1988; Gerad and Zak 1995).

Another index that indicates powder flow property is the Hausner ratio. Values less than 1.25 indicate good flow, while those greater than 1.25 indicate poor flow properties (Fonner et al. 1966). The Hausner ratios of extracts A, B and C were found to be 1.42, 1.27 and 1.25, respectively, indicating that all three extracts exhibit poor flow properties.

Angle of repose and flow rate are factors that are commonly considered to evaluate powder flow properties. As a correlation between results of these two methods does not always exist (Danish and Parrott 1971; Gold et al. 1966), both properties were determined. The angles of repose of extracts A, B and C were found to be 43.7°, 46.0° and 44.6°, respectively. Moreover, none of the three extracts would flow through a funnel of aperture size 10 mm under gravitational force. The results of both tests indicated that all the extracts exhibited poor flow properties. A number of reasons could be given for the poor flow properties of the extracts including particle shape and surface texture. As shown in Fig. 2 and 3, the crude extracts are composed of rough irregular particles, which can present more points of contact and hence higher friction resulting in less free flow. Another factor is the particle size and size distribution. The extracts are composed of fine particles (Fig. 1) with a large surface area and hence more contact points. Furthermore, fine particles lead to packing and powder densification resulting in poor powder flow properties.

Compactibility is defined as the ability of a powder bed to cohere into and form a compact mass. Compactibility is usually described in terms of tablet strength as a function of applied compaction stress (Gerad and Zak 1995). Fig. 7 shows the compaction profiles of extracts A, B and C. Extract C showed higher compactibility than extracts A and B. This high compaction may be attributed to the presence of some oils and fats remaining from the defatting process, which may contribute to the binding properties of extract C. The compaction profiles extracts A and B showed different profiles that cross over as the compression force increases. The difference in compaction profiles between the crude extracts may be due to variation of their composition, for instance the amount of extraneous material, which probably resulted in different compaction mechanisms. However, further studies have to be conducted to substantiate this assumption.

From the foregoing, it can be concluded that the standardised extraction method developed (defatting process, selected solvent systems and extract drying methods) provides an extract of *G. lotoides* with maximum yield of saponins. The physicochemical properties of the powdered extracts, such as particle size and size distribution, morphology, water sorption pattern, densities, flow properties and compaction profiles, depend on the nature of the extracting solvents and the drying methods employed.

3. Experimental

3.1. Materials

Fruits of *G. lotoides* were purchased from the local market, 'Merkato' in Addis Ababa, Ethiopia. Hexane, methanol, n-butanol, diethyl ether, potassium chloride, sodium chloride, sodium nitrite, sodium bromide ($NaBr \cdot 2H_2O$), potassium carbonate ($K_2CO_3 \cdot 1.5\,H_2O$), magnesium chloride ($MgCl_2 \cdot 6\,H_2O$), formamide, Hydranal® composite 2, sodium tartarate dihydrate, magnesium stearate and silicon dioxide were all of pharmaceutical grade (Merck, Darmstadt, Germany).

3.2. Extraction and purification of the seeds of G. lotoides

The extraction and purification of saponins from the seeds of *G. lotoides* was carried out as reported elsewhere (Endale 2000). In this method, the n-hexane defatted powdered seeds were extracted with various concentrations of methanol in water. The extracts were filtered, concentrated under reduced pressure and dried. The dried extracts were dissolved in water and partitioned between an equal volume of n-butanol. On separation, the n-butanol fraction was taken, dried and then dissolved in a small volume of methanol and added to a large volume of diethyl ether. The precipitate formed, containing mainly saponins, was separated and dried. This extract is referred to as purified extract.

3.3. Standardisation of the extraction method

3.3.1. Defatting and determination of total fat content

The outer pods of *G. lotoides* were separated from the fruits and the seeds were powdered. 5 g of the powdered seeds were defatted three times with 50 ml of n-hexane using an Ultura-Turrax mixer (T25, Janke & Kunkel, GmbH & Co. KG, IKA®-Labortechnik, Staufen i. Breisgau, Germany) at 9500 rpm for 10 min. The amount of fat removed at each defatting step was determined and compared to the total fat content of the seeds. Total fat content of the seeds of *G. lotoides* was determined by defatting with a large volume of hexane, i.e. 5 g of the seeds with 100 ml of n-hexane, five times until the final n-hexane extract became colourless.

3.3.2. Determination of solvent system for a maximum yield

Different concentrations of methanol in water (i.e., from 0 to 100% methanol) were examined for their extracting power. The required amount of silicon dioxide anti-foam emulsion and centrifugation at 2500 rpm for 4 min were utilised for solvent systems containing 50% methanol and below during Ultura-Turrax extraction and liquid-liquid extraction with n-butanol, respectively. The amounts of crude extract, n-butanol fraction and purified extract obtained were determined as described elsewhere (Endale 2000).

3.3.3. Determination of total saponin contents of the seeds of G. lotoides

5 g of the defatted seeds were extracted ten times repeatedly, each with 100 ml of 80% methanol, (i.e. a total of 1 l) until the final filtrate appeared colourless. The filtered extracts were combined, concentrated under reduced pressure at 40 °C and dried to provide the crude extract. The procedure employed for further treatment of the crude extract to yield an n-butanol fraction and subsequently a purified extract is described elsewhere (Endale 2000).

3.3.4. Extract drying methods

The concentrated aqueous methanol extracts were dried using a freeze dryer (LYOVAC GT2, FINN-AQUA® GmbH, Hürth, Germany) or oven (Heraeus Noblelight GmbH, Reinhaud-Heraeus, Germany) attached to a vacuum pump.

3.3.4.1. Freeze-drying method

The concentrated extract was first cooled to −70 °C for 24 h in a separate deep-freezer (GFL®, Gesellschaft für Labortechnik mbH, Burgwedel, Germany) and then dried in a freeze drier for 48 h using a heating programme that ranges from −30 °C to 20 °C. The freeze-dried extracts were kept in a desiccator.

3.3.4.2. Vacuum oven-drying method

The concentrated extract was dried to a semi-solid mass in a forced air oven (Type: ULE 700, Memmert GmbH, Schwabach, Germany) and then dried overnight in an oven attached to a vacuum pump (40 °C and −0.9 Bar).

3.4. Characterisation of the dry crude extracts

3.4.1. Particle size analysis

The extracts were first powdered in a mortar and pestle and then sieved until all particles passed though a 355 μm sieve. Particle size distributions of the 60%, 70% and 80% methanol powdered crude extracts (extract A, B and C, respectively) were measured using a Sympatec Laser Diffraction Spectrometer (Sympatec GmbH, Clausthal-Zellerfeld, Germany). A 200 mm focal length lens, a pressure of 4 bar and a measuring time of 5 s were used.

3.4.2. Morphology

The surface structures of the powdered crude extracts obtained by vacuum oven and freeze-drying and the purified extracts were investigated using a scanning electron microscope. Particles were sputtered with gold (Sputter Coater Typ E 5100, Bio-Rad GmbH, Munchen, Germany) and scanning electron micrographs were taken with a DSM 940 apparatus (Carl Zeiss, Oberkochen, Germany). The accelerating voltage and the magnification are shown on the photographs.

3.4.3. Water uptake

The water sorption patterns of the crude extracts, n-butanol fractions and purified extracts were determined using a computerised analyser (Krüss Processor Tensiometer K12, version 4.04, Krüss GmbH, Hamburg, Germany). Different relative humidities (33%, 44%, 59%, 65%, 75% and 85%) were prepared using saturated salt solutions of potassium chloride, sodium chloride, sodium nitrite, sodium bromide ($NaBr \cdot 2\,H_2O$), potassium carbonate ($K_2CO_3 \cdot 1.5\,H_2O$) and magnesium chloride ($MgCl_2 \cdot 6\,H_2O$), respectively. A powder bed of approximately 1 g of the extract was prepared on a balance hung in the humidity chamber. The weight gained per unit time was recorded. All the extracts were kept in a desiccator over silica gel for three days before conducting the experiment.

3.4.4. Water content determination

The moisture contents of the dry extracts were measured using a computerised Karl Fischer water-titration apparatus (Deutsche Metrohm GmbH & Co., Filderstadt, Germany). In this, 35 mg of the crude extracts of *G. lotoides* seeds were dissolved in methanol : formamide (2 : 1) and titrated with Hydranal® composite 2, (one – component reagent for volumetric Karl Fischer titration). The amount of Hydranal® composite 2 consumed was noted and the water contents of the extracts were calculated using a programme called "702 SM Tritrion". Standard sodium tartarate dihydrate, which contains 15.66% water was used as standard.

3.4.5. Densities

3.4.5.1. Bulk density

100 g of the dry extracts were poured into a 250 ml glass measuring cylinder and the volume was noted. The bulk densities were determined from known mass and bulk volume.

3.4.5.2. Tapped density

100 g of the dry extracts were poured into a 250 ml glass measuring cylinder, and tapped 1250 times using an Engelsmann tap meter (JEL ST 2, Engelsmann AG, Ludwigshafen, Germany) and the volume was noted. The tapped densities were determined from known mass and tapped volume.

3.4.5.3. True density

An air comparison pycnometer (Beckman GmbH, 8000 Munich, Germany) was used to determine the true densities of the powdered extracts. Atmospheric air was used as a comparison gas.

3.4.6. Powder flow properties

3.4.6.1. Angle of Repose

The angle of repose was determined by pouring 100 g of the dry extract through a funnel of 10 mm aperture size, measured at 75 mm height from the base, into a 10 cm diameter plate placed below the tip of the funnel.

3.4.6.2. Flow rate

The flow rate of the dry extracts was determined by pouring 100 g of the powdered extracts through a funnel of aperture size 10 mm with a closing end. The amount of extract passing per unit time under gravitational force was recorded.

3.4.7. Compactibility of the extracts

The crude extracts and 0.5% magnesium stearate were mixed in a Turbula mixer (Turbula T2C, W. Bachofer AG, Basel, Switzerland) for 5 minutes at 42 rpm. The mixtures were then compressed at various compression forces (4, 7, 10, 17, 21 and 27 kN) using an instrumented eccentric tablet machine (Korsch EK 0, E. Korsch GmbH, Berlin, Germany) fitted with 12 mm flat-faced punches.

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