Impact of Drying Methods and Extraction Solvents on the Steroidal Saponins Content of *Tribulus terrestris* Grown in the Peshawar Valley of Khyberpakhtunkhwa, Pakistan

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Abstract: The experiments were conducted to see the impact of drying methods and extraction solvents on the yield of steroidal saponins of *Tribulus terrestris*. The plant was dried by three different drying methods namely, solar collector drying, open sun drying and shade drying. After drying different levels (25-100%) of extracting solvent in the form of ethanol, methanol and distilled water were used for extraction. Soxhlet apparatus was used for extraction, while the gas chromatography apparatus was used in the experiment for detecting steroidal saponins in *Tribulus terrestris*. After extraction, four saponins identified were Tigogenin, Gitogenin, Hecogenin and Neohecogenin. The maximum yield of 61.2% was recorded for flat plate solar drying with 75% ethanol solution, followed 49.5% in shade drying with the same extraction solvent level. The lowest value of 3.1% yield was recorded for distilled water with open sun drying method. It was concluded that Both the drying methods and extraction solvent have a direct effect on the yield of steroidal saponins extracted from *Tribulus terrestris*. A maximum yield of almost 60% saponins may be achieved, if *Tribulus terrestris* is dried using a flat plate solar collector and extorted with 75% ethanol solution using GC-MS technique. Open sun drying minimizes saponins in *Tribulus terrestris*, while distilled water is the worse extracting solvent for extraction of steroidal saponins from *Tribulus terrestris*.

Key words: Flat plate solar collector, gas chromatography, soxhlet extortion, steroidal saponins, *Tribulus terrestris*

INTRODUCTION

Tribulus terrestris (Zygophyllaceae) known as puncture vine or devil weed is herbaceous and mat forming plant used for centuries, as a source of biomedicines. It is the most common Rabi crop weed found in warm, dry tropics of the world. The early Egyptians, Babylonians, Samaritans, Greeks, Romans, Indians and Chinese all used *Tribulus terrestris* in their system of medicines. It possesses special nutritional and healing powers that often go beyond chemotherapy (Hashim *et al.*, 2014). It contains a number of steroidal saponins and is used for the treatment of many diseases like infertility, cardiac, muscle building, conditioning and treatment of some other microbial diseases (Al-Bayati and Al-Mola, 2008). Besides saponins it also contains flavonoids, alkaloids, lignin amides and cinnamic acid amides. The amount of metabolites depends on the part of

the plant, which is used. The fresh juice of the aqueous extract of the whole plant contains inorganic nitrates, most likely potassium nitrate (Huang *et al.*, 2002). The fruit of *Tribulus terrestris* is rich with alkaloid, resin, fat, ascorbic acid, minerals and essential oils. It also contains a rich amount of iron and zinc, thus used for infertility in men and women. The difference in saponins content and composition is observed in different regions of the world (Bedir *et al.*, 2002; Chen *et al.*, 2013).

To extract the saponins from the plant, it is dried in either open sun or shade with high air velocities and after drying the saponins are extracted using different levels of ethanol and methanol as steroidal saponins are highly polar in nature (Ganzera *et al.*, 2001). During sun or shade drying, about 20-30% of saponins are lost due to the uncontrolled drying environment and degradation by direct Ultraviolet light rays (Sisto *et al.*, 2012). The present research aims to dry *Tribulus terrestris* in a

controlled drying environment using flat plate solar collector and study the effect of different drying methods, i.e., conventional including, open sun and shade drying and controlled convective drying by using solar collector, on the percentage of overall extracted saponins. The novelty of the research is to find the amount and composition of different saponins in the *Tribulus terrestris* grow in the Peshawar valley of the Khyber Pakhtunkhwa province of Pakistan.

MATERIALS AND METHODS

Plant materials: *Tribulus terrestris* (puncture vine) is the local and very common weeds of Peshawar Valley found in Rabi crops locally called as Markondi plant. Only ripe and good quality, weeds free from any spot, damage or foreign material was collected for the experiment (Sun *et al.*, 2003; Muthana, 2007) from the Newly Developmental Farm of the Agricultural University of Peshawar KPK, Pakistan.

Experimental setup: The experiment consists of three different drying setups. Drying by solar collector which is the main setup as shown in Fig. 1, consists of an indirect forced convection solar dryer with a flat plate solar collector, a circulation fan and a cabinet dryer. The drying was done by the procedure given by Hanif *et al.* (2013). The second setup as shown in Fig. 2 was to dry the plant

parts in open sun. The plant material was spread over the concrete floor on an area of 1.10 m² and dried in the sun (Kostova *et al.*, 2002; Saleh *et al.*, 1982). The third setup as shown in Fig. 3 was drying the plants in a shade under a velocity of 3.11 m sec⁻¹. The drying was performed in a dark room. Plant materials were dried until the moisture content reached <5%. The data of moisture in the plant were recorded after each hour of drying (Tomowa *et al.*, 1974; Kostova and Dinche, 2005).

Extraction of essential volatiles and GC-MS analysis: Soxhlet apparatus (Fig. 4) was used for extraction was considered as the reference technique. Exhaustive Soxhlet extraction was performed using a classical Soxhlet apparatus with an accurate measure of 1.0 g of *Tribulus terrestris* powder for 24 h. Extraction was performed with different levels of ethanol, methanol (25, 50, 75, 100%) and a single dose of distilled water as the extracting solvent. The extraction was conducted for 8 h for three times. Each time, the prior extract was removed and then 50 mL fresh ethanol was added. Finally, all the three extracts were combined and evaporated under vacuum. The extraction yield of total four saponins was defined as follows (Neychev *et al.*, 2007):

$$\text{Yield (\%)} = \frac{\text{Mass of saponin in one - step extraction solution}}{\text{Sum of the mass of saponins in sample}} \times 100$$

Fig. 1: General view of the solar dryer trays for drying purpose in the solar collector's dryer

Fig. 2: Open sun drying of *Tribulus terrestris*

Fig. 3: Shade drying of *Tribulus terrestris*

Fig. 4: Soxhlet apparatus used for extraction of volatiles in *Tribulus terrestris*

Fig. 5: Gas chromatography apparatus used in the experiment for detecting steroidal saponins in *Tribulus terrestris*

The mass of saponins in the extraction solution (one-step extraction) was analyzed by GC-MS (Fig. 5). The sum of the mass of saponins in the sample was determined by analysis of the total extraction solutions by continuous Soxhlet extraction for 24 h. Column flow was volumetric at 1.50 mL min^{-1} using ultra purified helium (99.999%) as the carrier gas. The splitless injection port was maintained at 250°C . The Injection was in split mode with a split ratio of 40:1. The transfer line temperature was set at 280°C . The mass analyzer was set at 70 eV, electron impact source temperature of 220°C . Electron-multiplier voltage of 1588 mV and solvents delay of three minutes. All data were obtained by collecting the full-scan mass spectra within the scan range of 50-600 amu. The temperature program was as follows: initially from $200\text{--}280^\circ\text{C}$ at ramp rate of $25^\circ\text{C min}^{-1}$ and from $270\text{--}280^\circ\text{C}$ at ramp rate of 1°C min^{-1} (Xu *et al.*, 2008, 2010a).

RESULTS AND DISCUSSION

Meteorological data: The meteorological data on the site are given in Fig. 6. The ambient temperature (T_a) varied between 27 and 40°C during the experimental period (May, 2014). The temperature for the open sun system remain the same as the ambient temperature, while for the shade it is 2% less than that of ambient. The solar collector allowed increasing the air temperature (T_{out}) up to 27°C above the ambient temperature, depending on the mass air flow rate and mainly of the solar flux on a tilted collector. During the complete drying period, the solar flux on a tilted collector remained above 550 W m^{-2} , with a peak value of 712 W m^{-2} at around (12.00 noon). The temperature and relative air humidity of the drying air at the inlet of the drying chamber were also registered, which are presented for some runs. The drying air temperature at the inlet of the drying chamber kept at the

Fig. 6: Meteorological data in Peshawar (Pakistan). Solar flux on a tilted collector, Relative humidity and temperature of the drying air recorded in May 2014

values of the interval $50\text{--}64^\circ\text{C}$ with variations of $\pm 1^\circ\text{C}$. The ambient relative humidity varies from 51-76% with variations $\pm 2\%$. The drying temperature is raised to give a lower relative humidity of drying air. The relative humidity of the drying air at the inlet of the dryer is less than $5\pm 2\%$. The lower the relative humidity of drying air supplied to the drying chamber is the better the drying rate, as the less humid air can tend to pick up moisture more quickly and easily from the product surface than the more humid air. In addition to that, Demir *et al.* (2007) stated that a solar dryer, which provides a temperature in the range of $45\text{--}65^\circ\text{C}$ and a relative humidity of drying air less than 20% is favorable for drying any agricultural product. In this study, the solar dryer took only 9 h for reducing the moisture content of white mulberry inside the dryer from 76-9% (wb). During the experiment, the drying air passing through the product at a mass flow rate of 1.5 kg min^{-1} with the accuracy of $\pm 0.1 \text{ m sec}^{-1}$, whereas the relative humidity is below 10% and the temperature varies between 50 and 65°C .

Drying curves: The drying curves by the three setups is shown in Fig. 7. The graph shows that flat plate solar collector took 9 h to dry the plant material, convective shade drying took 19, while the conventional sun drying system took 27 h of drying period to dry the plant. The drying time is reduced from 27-9 h, which showed that flat plate solar collector save more time of drying as compared to conventional drying of *Tribulus terrestris* by the open sun drying system. The results are in accordance with the findings of Hanif *et al.* (2013), who reported that flat plate solar drying systems facilitates drying, increase quality and reduce drying time. These results are also in accordance with the findings of Sisto *et al.* (2012).

Fig. 7: Drying curves of the three drying setup of *Tribulus terrestris*

Fig. 8: Total ion mass chromatogram of *Tribulus terrestris* by GC-MS. Representing 1. TG, 2. GG, 3. HG and NG

Identification of saponins: With the reported chromatographic conditions (Li *et al.*, 2009) a capillary column of HP-5MS was particularly useful for the separation of saponins. Absolute separation of the analytes was attained in 22 min (Fig. 8). The Total ion mass chromatogram of saponins obtained was differentiated by the manifestation of Molecular Ions (M1) and an ion at the base peak. Four different steroidal saponins from the extracts were identified, who targets matched to 99% fit with the standard steroidal saponins provided by the Pakistan Academy of Sciences (PAS).

Recognition of steroidal saponins in the analytes was based on the relationship of retention time and mass spectra to the genuine compound. Identification of the four saponins was, according to the molecular weight and standard spectra of the Standards. The four saponins identified are; 1. Tigogenin (TG-M+416 amu), 2. Gitogenin (GG-M+432 amu), 3. Hecogenin (HG-M+430 amu) and 4. Neohecogenin (NG-M+430 amu). The structure of all the four saponins is as shown in Fig. 9. The formulae of each structure of four steroidal saponins identified by GC-MS in *Tribulus terrestris* is given in Table 1. The same type of saponins were resulted

from the extracts of *Tribulus terrestris* used in experiments by Bedir *et al.* (2002), Chen *et al.* (2013), Ganzera *et al.* (2001) and Huang *et al.* (2002).

The impact of extraction solvent and drying methods on the yield of steroidal saponins extracted from *Tribulus terrestris* is given in Table 2. The statistical analysis of the data showed that both the factors have a significant effect ($\alpha < 0.001$) on the extracted yield of steroidal saponins from *Tribulus terrestris*. The maximum yield of 61.2% was recorded for flat plate solar drying with 75% ethanol solution, followed 49.5% in shade drying with the same extraction solvent level. The lowest value of 3.1% yield was recorded for distilled water with open sun drying method. The means comparison of drying methods showed that highest yield of 32.0% was recorded for flat plate solar drying system followed by shade drying with a value of 28.5%, while the lowest yield of 25.5% was recorded for the open sun drying system. Similarly the means comparison of extracting solvent solution levels showed that highest yield of 52.3% was recorded for ethanol 75% followed by 44.2% in 100% ethanol. The lowest yield of 3.9% was recorded for distilled water. It is clear from the results that saponins

Fig. 9: Four steroidal saponins extracted from *Tribulus terrestris* found in the Peshawar Valley of KPK. Pakistan, (a) Tigogenin (b) Gitogenin (c) Hecogenin and (d) Neohecogenin

Table 1: Formulae of each structure of four steroidal saponin identified by GC-MS in *Tribulus terrestris*

Peak No.	Retention time (min)	Fit with the standard (%)	Name of steroidal saponin	Formula of steroidal saponin	Fraction	±SD
1	11	99	Tigogenin (TG)	25 R-5 α -Spirostan-3 β -ol	13.89	±1.15
2	15	99	Gitogenin (GG)	25 R-5 α -Spirostan-2 α ,3 β -diol	7.82	±1.01
3	16	99	Hecogenin (HG)	25 R-5 α -Spirostan-12-one	25.76	±1.62
4	17	99	Neohecogenin (NG)	25 S-5 α -Spirostan-12-one	7.93	±0.91

Table 2: Mean comparison ($\alpha < 0.001$) of drying methods and extraction solvent on the yield of steroidal saponins extracted from *Tribulus terrestris*

Extracting solvent levels	Drying methods			Mean
	Shade drying	Open sun	FPS collector	
Ethanol 25	17.3	13.2	19.1	16.5 ^d
Ethanol 50	30.2	26.5	35.4	30.7 ^c
Ethanol 75	49.5	46.3	61.2	52.3 ^a
Ethanol 100	44.2	43.8	45.1	44.4 ^b
Methanol 25	22.9	20.9	23.4	22.4 ^d
Methanol 50	35.2	31.0	35.9	34.0 ^c
Methanol 75	28.7	24.1	31.2	28.0 ^d
Methanol 100	24.3	20.3	29.9	24.8 ^d
Distilled water	4.5	3.1	7.2	4.9 ^e
Mean	28.5 ^a	25.5 ^b	32.0 ^a	

LSD for drying methods = 8.45, LSD for extracting solvent = 2.92, Means followed by different alphabets are significantly different from each other

are highly polar compounds and their extraction is highly effected by the use of extracting solvent. Ethanol and methanol gave good yields because they are non polar where is the yield was minimum for distilled water because of the polarity effect of the two solvents. These results are in accord with the findings of Kostova and Dinche (2005), Muthana (2007) and Xu *et al.* (2008, 2010b).

CONCLUSION

Both the drying methods and extraction solvent have a direct effect on the yield of steroidal saponins extracted from *Tribulus terrestris*. A maximum yield of almost 60% saponins may be achieved if *Tribulus terrestris* is dried using a flat plate solar collector and extorted with 75% ethanol solution using GC-MS technique. Open sun drying minimizes saponins in *Tribulus terrestris* is while distilled water is the worse extracting solvent for extraction of steroidal saponins from *Tribulus terrestris*.

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