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Copacetic herbal extraction techniques and diabetes induction methods for developing countries

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ABSTRACT

Extraction is an empirical exercise for the discovery of bioactive constituents from the plant materials. Selection of a suitable extraction technique is also important for the standardization of herbal products and to eliminate unwanted material by treatment with a selective solvent known as "menstrum". Traditional conventional methods such as maceration, rotary evaporation, soxhlation, percolation, lyophilization, infusion, decoction etc are more cheap and appropriate for developing countries rather than fast and environmentally friendly green extraction techniques like ultrasound assisted solvent extraction, microwave assisted solvent extraction and supercritical fluid extractions for phytochemical processing of medicinal plants. Moreover, plant-derived phytochemicals have established their role for possible use in diabetes treatment which is the world's largest endocrine disease associated with increased morbidity and mortality rate. Several experimental models are used to study pathophysiological factors involved in diabetes and to assess the efficacy of herbal antihyperglycemic agents. Today different strains of laboratory animals are available and therapeutic studies on diabetes are mostly carried out on these models. In animals, it can be induced by partial pancreatectomy or by the administration of diabetogenic drugs such as alloxan, streptozotocin, ditizona and anti-insulin serum. Hence, the review is aiming to draw attention of the relevant researchers to the convenient use of suitable extraction technique and diabetogenic drugs for developing countries.

Key Words: Copacetic, Herbal extraction, Diabetes, Induction method and Developing countries

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I. Introduction

Plants are still important sources of medicines, especially in developing countries that still use plant-based traditional medicine for their healthcare (Salim et al., 2008). Plants are an important source of bioactive molecules for drug discovery. Isolated bioactive molecules serve as starting materials for laboratory synthesis of drugs as well as a model for the production of biologically active compounds (Dhanani et al., 2013). Phytochemical processing of raw plant materials is essentially

required to optimize the concentration of known constituents and also to maintain their activities (Satyanshu, 2015). Extraction is the separation of medicinally active portions of plant tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or in dry powder form (after removing the solvent), & are intended for oral or external use (Tiwari et al., 2011). The purpose of standardized extraction procedures for crude drugs (medicinal & aromatic plant parts) is to attain the therapeutically desired portions (Tiwari et al., 2011). Various extraction techniques most commonly used include conventional techniques such as maceration, percolation, infusion, decoction, hot continuous extraction etc. Recently, alternative methods like ultrasound assisted solvent extraction (UASE), microwave assisted solvent extraction (MASE) and supercritical fluid extractions (SFE) have gained increasing interest during the last three decades though these technologies are quite expensive (Saurabh et al., 2015).

As about 50% of the Bangladeshi populations are at risk of diabetes each year, the majority of people especially in rural areas, use herbal medicine alone or alongside prescription drugs for diabetes management (Amin et al., 2015). Experimental models play an important role in understanding such a disease, which is treatable only. In animals, it can be induced by partial pancreatectomy or by the administration of diabetogenic drugs such as alloxan, streptozotocin, ditizona and anti-insulin serum (Carvalho et al., 2003). These agents selectively destroy the β -cells of islets of Langerhans. In most laboratories this diabetogenic drug induced experimental model used more conveniently as alternative model in therapeutic studies on diabetes (Carvalho et al., 2003). This review provides useful resources to draw attention of the relevant researchers on the available phytochemicals extraction techniques and diabetes induction methods and also give importance on their more rational use in the developing countries like Bangladesh.

II. Methodology

This review article has been prepared from secondary study materials such as published journal articles, review papers, conference papers & reports, technical and theoretical notes, reports, books and relevant information found from various online sources.

III. Discussion

Extraction process

There are a number of extraction techniques used by the scientists in the developing countries, given in a flow chart (Figure 01). These are discussed below.

Rotary evaporation: Plants parts are first collected and identified by the specialist. Then they are washed carefully and dried immediately either in an artificial environment at low temperature (50-60°C) or dried preferably in shade so as to bring down the initial large moisture content to enable its prolonged storage life. The dried portions are then grinded using grinder to make fine powder. The powder is then dissolved in alcohol. The suspension is filtered with filter paper and dried by rotary evaporator and alcoholic extract is obtained. It is finally stored in airtight container in refrigerator for further use (Kulasekharam, 2014; Bhuyan et al., 2010; Nahar et al., 2010; Sikder et al., 2011; Sophia and Manoharan, 2007; Varma et al., 2009; Zulfiker et al., 2011).

Soxhlation: Collected and identified plants parts are carefully washed and dried in sunlight. The dried portions are then grinded to make fine powder. The powder is packed separately in the Soxhlet apparatus and extracted with alcohol, until the completion of the extraction. The extract is filtered while hot and the resultant extract is distilled in vacuum under reduced pressure in order to remove the solvent completely and later dried in a desiccator. After that alcohol extract is kept in air tight container for further study (Farswan et al., 2009; Maurya et al., 2011; Prasad et al., 2014; Sachan et al., 2009; Samyial et al., 2014; Singh and Gupta, 2007; Vivek et al., 2010).

Percolation: The experimented parts are first washed well and dried at room temperature and coarsely powdered. The powder is extracted with hexane to remove lipids. It is then filtered and the

filtrate is discarded. The residue is successively extracted with methanol using cold percolation method. The extract is stored at -70°C (Herroor et al., 2013; Mastan et al., 2009; Patil et al., 2010).

Lyophilization: The plant parts are washed with clear water and then ground for 10 min in a mixer along with the distilled water (500 ml). It is allowed to stand overnight and then filtered through several layers of muslin cloth. The whole procedure is carried out in cold condition at 4°C . The filtrate is centrifuged in a refrigerated centrifuge at 10,000 rpm. The supernatant is lyophilized to get a thick paste of water extract (Ramana et al., 2011; Rekha et al., 2008).

There are four (4) common extraction processes as described below:

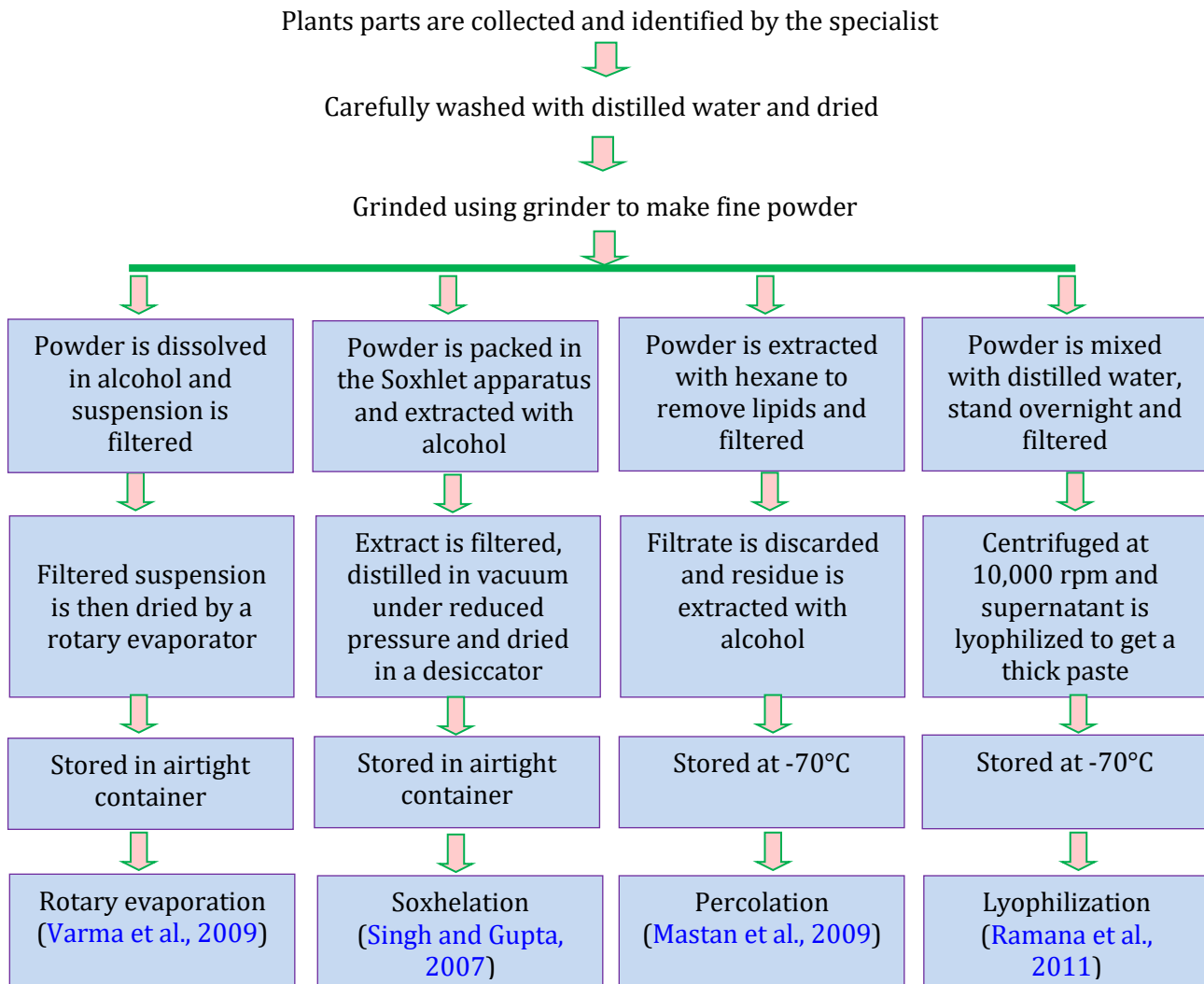


Figure 01. Brief illustration (flow chart) of the extraction processes.

In the developing countries like Bangladesh, rotary evaporation method is chiefly used for extraction as it allows for quick, gentle evaporation of solvents; even it is relatively easier for the inexperienced users. New techniques such as supercritical fluid extraction (SFE), microwave-assisted extraction, solid phase extraction, chromatographic fingerprinting and marker compound analysis, chromatographic techniques (liquid chromatography, gas chromatography, supercritical fluid chromatography) are highly expensive but are particularly useful when one has many samples to do in parallel.

Diabetes induction methods

Different models to induce experimental diabetes mellitus are:

- a) Alloxan induced diabetes

- b) Streptozotocin induced diabetes
- c) Other diabetogenic compounds
- d) Pancreatectomy in dogs
- e) Hormone induced diabetes
- f) Insulin deficiency due to insulin antibodies
- g) Virus induced diabetes (Grover et al., 2011)

Table 01. Diabetes induction methods by using Streptozotocin

Dose	Animals used	References	Route
1. Streptozotocin in physiological saline @			
▪ 35 mg/kgbw	Sprague Dawley rat	Jonnalagadda et al., 2013	Single intra-peritoneal injection
▪ 45 mg/kgbw	Wister albino rat	Kulasekharam, 2014 ; Maurya et al., 2011	
▪ 50 mg/kgbw	Sprague-Dawley rat Wister albino rat	Ramana et al. 2011 Sridhar et al., 2005 ; Varma et al., 2009	
▪ 55 mg/kgbw	Wistar rat	Rekha et al., 2008	
▪ 90 mg/ kgbw	Long-Evans rat Wistar albino rat	Bhuyan et al., 2010 Farswan et al., 2009	
2. Streptozotocin in physiological saline @			
▪ 55 mg/kgbw	Wistar rat	Ahmed and Urooj, 2009	Single intramuscular injection
3. In physiological saline			
▪ Nicotinamide @ 120 mg/kgbw + Streptozotocin @ 60 mg/kgbw	Wistar albino rat	Samyal et al., 2014 ; Sarma, 2014	Single intra-peritoneal injection

Table 02. Diabetes induction methods by using Alloxan Monohydrate

Dose	Animals used	References	Route
1. Alloxan monohydrate in physiological saline @			
▪ 60 mg/kgbw	Wistar albino rat	Singh and Gupta, 2007	Single intravenous injection
▪ 150 mg/kgbw	Sprague-Dawley rat	Sachan et al., 2009	
2. Alloxan monohydrate in physiological saline @			
▪ 100 mg/kgbw ▪ 110 mg/kgbw ▪ 120 mg/kgbw	Albino rat	Mastan et al., 2009 Nahar et al., 2010 Nair and Santhakumari, 1986 ; Vivek et al., 2010	Single intra-peritoneal injection
▪ 150 mg/kgbw	Rats Long Evan's rat Albino Wistar rat Swiss albino mice	Heroor et al., 2013 Sikder et al., 2011 Sophia and Manoharan, 2007 Zulfiker et al., 2011	
3. Aqueous solution of Alloxan monohydrate			
▪ 80 mg/kgbw ▪ 110 mg/kgbw ▪ 150 mg/kgbw	Wistar albino rat Albino Wistar rat Swiss albino mice	Prasad et al., 2014 Patil et al., 2010 Sufiyan et al., 2014	Single intra-peritoneal injection

Animal models of diabetes are increasingly being used for pathophysiology and pharmacological studies of diabetes mellitus. Advantages of animal studies in the examination of alternative medicines and their efficacy include the ability to define experimental conditions more tightly and to undertake more detailed studies of the biologic effects of the agents being used (Emam, 2012). Streptozotocin

and Alloxan monohydrate-induced hyperglycaemia in animals are considered to be a good experimental model since they are less toxic than other chemical agents inducing diabetes. The methods for diabetes induction by using streptozotocin and alloxan monohydrate are summarized in (Table 01) and (Table 02). Other than streptozotocin and alloxan monohydrate, dithizone or goldthioglucose can be used as diabetogenic agents but those are costly and hardly available. Pancreatectomy and hormone induced diabetes are mostly suitable for dogs and cats. Other methods such as insulin deficiency due to insulin antibodies or virus induced diabetes are difficult to execute.

Streptozotocin is a naturally occurring chemical and carries a substantial risk of toxicity. Alloxan monohydrate is highly toxic to the liver and the kidneys. So the lowest dose i.e., @ 35mg/kgbw single, intra-peritoneal injection of streptozotocin and 6 mg/100 gbw single intravenous injection of alloxan monohydrate would be safer for diabetes induction in the animals.

IV. Conclusion

Despite all of the advances made by the pharmaceutical industry in the development of novel and highly effective medicines for the treatment of a wide range of diseases, there has been a marked increase in the use of herbal medicines even including the more affluent countries of the world (Doughari, 2012). For the separation of medicinally active portions of plant tissues, suitable extraction method is inevitable. Each method has its own advantage or disadvantage. Hence, to make the extracts available to the mass people, cheap and safe methods such as the rotary evaporation method may be appropriate in our country. On the other hand, diabetogenic drugs are easy to administer for diabetes induction than other known diabetes induction methods and the best known drug-induced diabetes model is the alloxan diabetes. Hence, the review will create some new ideas in the avenue of research and will offer better opportunities to the scientists to have a convenient and more suitable extraction technique and diabetes induction method.

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