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Design, Formulation and Evaluation of Oxymel Containing *Andrographis paniculata* Extract

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ABSTRACT

Introduction: *Andrographis paniculata* is medicinal plant of family Acanthaceae, consists of bitter principles and traditionally used in treatment of several gastrointestinal diseases. To mask its bitter taste, it could be formulated in honey based oral formulations like oxymel. Honey, as a saturated solution of various sugars, as per Ayurvedic system of medicine, could be consumed along with drug. **Aim:** This research attempt was aimed towards aqueous extraction of *A. paniculata* powder; formulation of oxymel by addition to honey and evaluation for different parameters. **Methods:** Oxymel was formulated as per procedure mentioned in United State Pharmacopeia for squill oxymel; and evaluated for pharmaceutical parameters those applied for oral syrups. **Results:** The oxymel formulated was dark brownish with agreeable odour and sweet taste. It was pourable with viscosity of 27.14 CP at 45.02 torque measured at 100 rpm while density was found to be 1.25 gm/ml. There was also ease in cap opening of its container, also no crystallization of honey was observed. Its andrographolide content was found to be 411.0 µg/ml. **Conclusion:** Bitters of *A. paniculata* have significant pharmacological activities in human being, if administered orally. To mask their bitter taste and facilitate their increase in absorption, *A. paniculata* can successfully be formulated in honey based oral formulation of oxymel.

Key words: Kalmegh, Andrographolide, Honey, Liquid dosage form, HPLC.

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INTRODUCTION

Any drug molecule, based on its dose, site and mechanism of action could be administered by oral route through any of solid unit dosage forms like tablets, capsules, pills, powders; biphasic systems like emulsion, suspensions or liquid dosage formulations like tinctures, elixirs, solutions, syrups and oxymels. Oxymels are the oral dosage forms prepared using honey as base and added with acetic acid. Sweet taste of honey fulfils the aim of taste masking of bitter drugs which are orally active. Squill Oxymel was one of the widely used formulations for appetite. Oxymel is an oldest dosage form that has been using in Europe, however in Ayurvedic system of medicine, honey is also used as '*anupana*', edible ingredient that is administered along with drug or meal or afterward, for its adjuvant action for the active drug molecule and taste masking of bitters.[1] As per Chinese system, ephedra extract can be administered after honey-frying processing.[2] Honey is a supersaturated solution of monosaccharide's, mainly fructose and glucose, containing also minerals, proteins, free amino acids, enzymes, vitamins and polyphenols mainly flavonoids.[3]*Andrographis paniculata* (Burm. f.) Wall. ex Nees (AP) (Figure 1), family Acanthaceae, commonly known as *Kalmegh* or *Kadu kirayat*, is medicinal plant traditionally and widely used across the world, mainly in Asian countries like India, Bangladesh, Pakistan and China.[4] *A. paniculata* is therapeutically well established and effectively used in treatment of dyspepsia,[5] diarrhea,[6] jaundice.[7] It also has anti-inflammatory[8] and anti-hyperglycemic[9] activities. On phytochemical analysis, so far, *A. paniculata* has been reported to contain bitter principles, andrographolide (Figure 2), isoandrographolide, 14-deoxy-11,12-dihydro-andrographolide, 14-deoxy-12-methoxy-andrographolide, 14-deoxyandrographolide and few specific flavones, andropaniculosin A, andropaniculoside A, wogonin, onysilin and isoswertisin.[10] Hence, it can be concluded that pharmacological actions exhibited by *A. panicuata* are because of these phytochemicals. As per Indian Pharmacopoeia, the plant is prominent in around 26 Ayurvedic formulations, indicating its significance. There are evidences where *A. paniculata* extract administered by oral route.[11] Tested *A. paniculata* extract for its beneficial effects on cognitive functions in streptozotocin-induced diabetic rats. Few researchers made formulations of *A. paniculata* extract.[12] formulated *A. paniculata* extract into tablets, prepared by wet granulation method and evaluated for their *in vivo* anti-malarial potential. In order to improve the oral bioavailability of andrographolide, Du *et al.* 2012 and Sermkaew *et al.* 2013 prepared andrographolide and *A. paniculata* extract - loaded liquid and solid self-micro emulsifying drug delivery systems for oral administration, respectively.[13,14] Moreover, pharmacokinetic and pharmacodynamic interaction of *A. paniculata* extract and andrographolide with etoricoxib after oral administration was also studied.[15] Considering the pharmacological importance of bitters present in *A. paniculata*, it must be formulated in modern dosage forms where its bitter taste could be masked. Hence, the present study was aimed at formulation of oxymel containing *A. paniculauta* extract and its evaluation for various pharmaceutical parameters.

MATERIALS AND METHODS

Materials

Andrographis paniculata powder, sample well identified by botanist, was procured from commercial supplier, Yucca labs, Mumbai. Acetic acid used was manufactured by Analab Fine Chemicals Ltd. Mumbai. Honey used in the formulation was supplied as brand Dabur honey.

Formulation of oxymel

Formulation of oxymel containing *Andrographis paniculata* extract was based on method described for squill oxymel in.[16] Firstly, *A. paniculata* powder was macerated with 33% acetic acid and purified water for 7 days with occasional agitation and boiling. Then, it was filtered and this filtrate was preliminary screened for recognition of class of phytochemicals present in it. It included detection of alkaloids, tannins, flavonoids, iridoids, triterpenoids by chemical tests based on colour change or precipitation by addition of specific reagent as specified[17] and its andrographolide content was quantified by HPLC method specified[18] Then, to every 3 parts of filtrate, 7 parts of honey were added, with constant agitation. The oxymel so prepared was then stored in dry place until further used for its evaluation.

Evaluation of oxymel

The resultant oxymel formulation was evaluated for different pharmaceutical parameters, those described for squill oxymel USP and those for oral liquid dosage forms like syrup.

Organoleptic evaluation

It included simple visual and sensory inspection of colour, odour and taste.

pH

The pH of oxymel was determined using pH meter, Equip-tronics model EQ-614.

Viscosity

Viscosity of oxymel was determined using Brookfield DV-E viscometer with spindle no. 61.

Density

Density as weight per ml was determined using pycnometer.

Crystallization evaluation

In order to evaluation of possible crystallization, the oxymel was placed in refrigerator for a period of a week and then examined for precipitation.

Cap locking

To evaluate cap locking, oxymel was filled in container, capped and placed in inverted position for a week. Then, ease in cap opening was checked.

Assay for acetic acid

About 20 ml of oxymel was diluted with 20 ml of carbon dioxide free water and titrated with 1 M sodium hydroxide, using phenolphthalein solution as indicator. Acetic acid content was determined on the basis of equivalence of each ml of 1 M sodium hydroxide to 60.05 mg of acetic acid.

Quantification of andrographolide/ content determination

The andrographolide content in 33% acetic acid and formulation were determined by reversed phase HPLC analysis as described[18] where 20 µL of sample was injected to C_{18} column through which binary solvent system of water and methanol (35:65) flowed at 0.7 mL/min and the analysis was carried out with UV-Vis detector at 223 nm.

Firstly, andrographolide content of *A. paniculata* extracted with 33% acetic acid was determined and it was added to prescribe quantity of honey to form oxymel. Then, andrographolide content of oxymel was determined.

RESULTS

The content of acetic acid and pH of oxymel were found to be 31.8 mg/ml and 5.04, indicating that during extraction of *A. paniculata* powder, some quantity of acid got neutralized. The presence of andrographolide was confirmed by brown/blue band at R_f value 0.5 while HPLC analysis of the filtrate carried out at given specifications, determined the concentration of andrographolide having retention time 7.15 min. It

Figure 1: *Andrographis paniculata* (Acanthaceae).

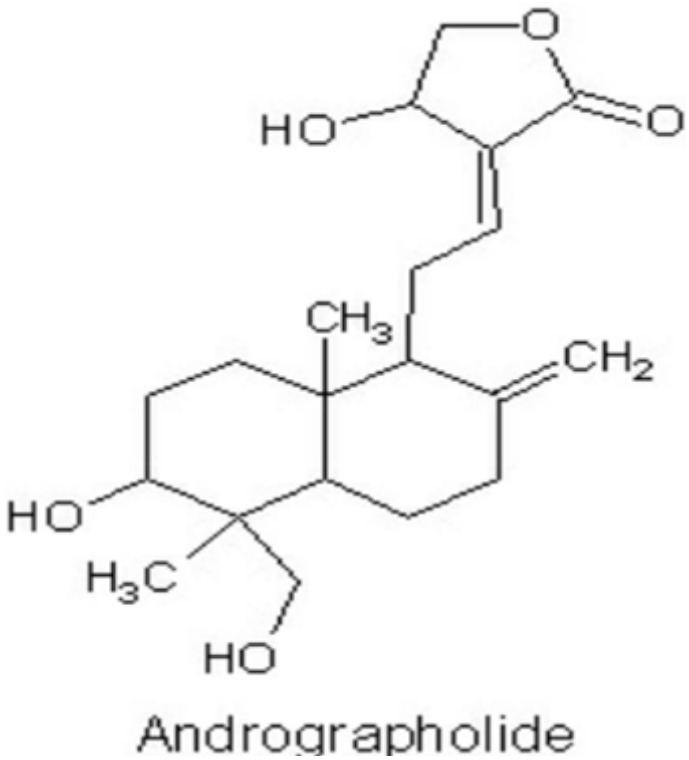

Figure 2: Structure of andrographolide.

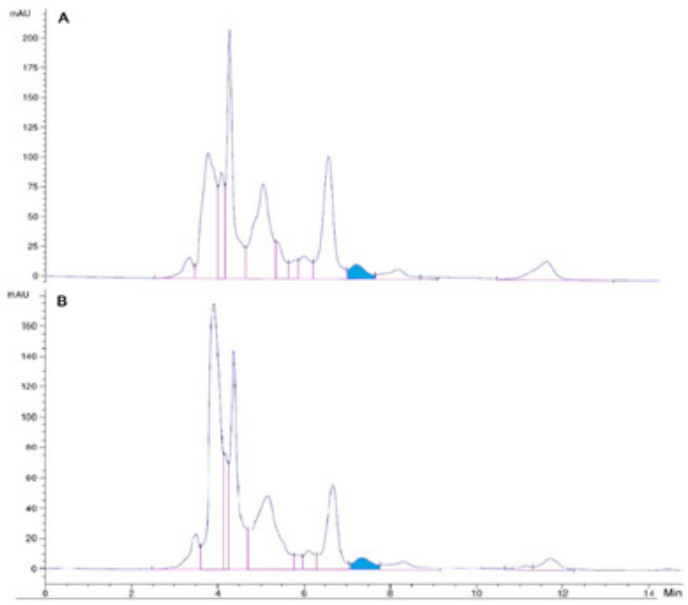

Figure 3: HPLC chromatograph determining andrographolide content (blue shaded peak areas) from, A: *A. paniculata* extract in 33% acetic acid; B: Oxymel formulated containing *A. paniculata* extract and honey.

was found to be 581.2 µg/ml (Figure 3A, Andrographolide content was indicated by blue shaded peak area in chromatograph).

The oxymel, formulated by USP based procedure, was dark brownish in color with agreeable odour and sweet taste. It was pourable from one container to another. Viscosity, as resistance to flow, of oxymel was determined as 27.14 CP at 45.02 torque measured at 100 rpm while density was found to be 1.25 gm/ml. It was also observed that oxymel sample did not get crystallized even after placed in refrigerator for a week. There was also ease in cap opening after placing in inverted position for a period of a week. By HPLC based analysis of oxymel, andrographolide content was found to be 411.0 µg/ml (Figure 3B, Andrographolide content was indicated by blue shaded peak area in chromatograph).

DISCUSSION

As such, andrographolide is insoluble in water but soluble in alcohols, pyridine, acetic acid and acetone. In neutral to basic pH, andrographolide is unstable and gets hydrolysed to an inactive product.[19] Hence, due to presence of acetic acid, it is expected that andrographolide gets solubilised and therefore acetic acid was assayed.

Andrographolide exhibit poor therapeutic application due to it's a low oral bioavailability (2.67%) The reasons encountered for this include site specific absorption from upper GI tract; pH dependent hydrolysis in weak alkaline environment of intestine; spontaneous metabolism to sulphate metabolite (14-deoxy-12-sulfo andrographolide), impermeable to intestinal wall; P-glycoprotein-mediated and biliary excretion and physical properties like high lipophilicity (log *P* value 2.632 ± 0.135) with low aqueous solubility (3.29 ± 0.73 µg/ml).[20]

It has also been observed that maximum plasma drug concentration (C_{max}) as a function of bioavailability of pure andrographolide is lesser (27.24±3.23 µg/mL) than that when present in *A. paniculata* extract which is administered in tablet form (35.22±3.54 µg/mL).[21]

Many pharmacologically active molecules get metabolised by phase I, mainly oxidation and phase II, conjugation with sulpho group reactions. However, both of these reactions are inhibited by flavonoids, mainly Galangin found in honey inhibits cytochrome P450-dependent mixed-function oxidases (CYPs) and sulphotransferases (SULTs).[22] Hence, it could be concluded that combination of honey with active principles, decreases their metabolism at least up to some extent.

CONCLUSION

The bitter principles of *A. paniculata* could be extracted using aqueous solution of acetic acid (33%). They exhibit significant pharmacological actions when administered orally. Brownish oxymel formulated with *A. paniculata* extract has agreeable odour and sweet taste. Its pharmaceutical parameters evaluated were in acceptable range. HPLC analysis determined the andrographolide content in extract and oxymel; and revealed that formulation of oxymel does not affect the bitters. Combination of bitters with honey decreases their metabolism.

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ABBREVIATIONS

***A. Paniculata*:** *Andrographis paniculata*; HPLC: High Performance Liquid Chromatography; **USP:** United State Pharmacopeia; **UV VIS Detector:** Ultraviolet Visible Detector; **R_f:** Retention factor; **GI:** Gastrointestinal; **CP:** Centipoise; **RPM:** Rotation per minutes.

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