KeAi CHINESE ROOTS GLOBAL IMPACT

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DESIGN AND DEVELOPMENT OF NEUTRITIVE AND SUPPLEMENTARY HERBAL DROPS FOR ANTI-DIABETIC ACTIVITY

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ABSTRACT:
This study aim to design develop and assess a herbal drops containing *salvia rosmarinus* for treating infection and diabetes. The selected herbal drugs are recognized for their safety and minimal side effects compared to allopathic medicines. The herbal drugs were processed into a powder and mixed to create an extract resulting in a medicated herbal drop. The prepared medicated herbal drops is characterized for appearance, taste, odour, boiling point, thin layer chromatography, gas chromatography and in vitro anti-microbial activity. The taste of the drug is bitter there is no use of any sweetener. The presence of phytochemical component in extract is analyzed by gas chromatography. Antimicrobial effectiveness of the formulations against *staphylococcus aureus and E coli* was evaluated using the cell diffusion assay. This research highlights the development of a drops with potential immune booster benefits, which has potential anti diabetic activity.

Keywords: Immune booster**,** Anti-diabetic, Herbal medicated drops, Rose merry leaves, Extract.

Introduction:

Extraction is the process for the isolation of the active ingredient from drug material. Medical plants are extracted and processed for direct consumption as herbal or traditional medicine or prepared for experimental purpose. The concept of preparation of medicinal plant for experimental purpose involves the proper and timely collection of the plant, authentication by an expert, adequate drying and grinding. This is followed by extraction, fractionation and isolation of the bioactive compound where applicable. In addition, it comprises determination ofquality and purity of bioactive compounds.

Commonly used methods in the extraction of medicinal plants are

1. Maceration
2. Infusion
3. Digestion
4. Percolation
5. Soxhlet

Diabetes is chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood glucose. Hyperglycemia, also called raised blood glucose or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's system, especially the nerves and blood vessels.

Diabetes mellitus has been classified into two types i.e, insulin dependent diabetes mellitus (IDDM, Type I) and non-insulin dependent diabetes mellitus (NIDDM, Type II). Type I diabetes is an autoimmune disease characterized by a local inflammatory reaction in and around islets that is followed by selective destruction of insulin secreting cells whereas Type II diabetes is characterized by peripheral insulin resistance and impaired insulin secreation. The presence of DM shows increased risk of many complications such as cardiovascular diseases, peripheral vascular disease, stroke, neuropathy, renal failure, retinopathy, blindness, amputations, etc.

Types of Diabetes Mellitus

The main types of Diabetes Mellitus are:

Insulin Dependent Diabetes Mellitus (Type I IDDM): This type of diabetes mellitus is also called autoimmune diabetes and previously known as juvenile-onset or ketosisprone diabetes. The individual may also seek with other autoimmune disorder such as Grave's disease, Hashimoto's thyorididitis and Addison's disease. Type I diabetes mellitus occurs mainly in children and young adults; the onset is usually sudden and can be life threatening.

Non-Insulin Dependent Diabetes Mellitus (Type II NIDDM): Type II diabetes mellitus is also known as adult-onset diabetes. The progressive insulin secretary defect on the backgroundof insulin resistance (American Diabetes Association, 2014). People with this type of diabetes frequently are resistant to the action of insulin. The long term complication in blood vessels, kidney, eyes and nerves occurs in both type and are the major causes of morbidity and death

Causes of Diabetes Mellitus:

Aging, Obesity, Insufficient energy consumption, Alcohol, Smoking

Symptoms of Diabetes:

Increased thrush, Frequently urination, Extreme hunger, Weight loss, Fatigue, Polyuria, polydipsia, glycosuria

Material and Method:

The part of plants of *salvia rosmarinus* were freshly collected from market. The plant were identified and authenticated by, Department of Botany, KBP College Pandharpur, India. The Plant materials washed and dried under the shed and converted into powder by mechanical grinding. Then powders were passed through sieve no. 16, and used for further study.

Phytochemical Screening-

In order to determine the presence and absence of primary and secondary metabolites,

phytochemical screening of plant extracts has been carried out using a standard procedure to verify the presence and purity of herbal medicinal products. Gas chromatography is carried out as per the standard procedure.

Preparation of drops:

Preparation of extract: Twig of the plant were air dried in shade and grind to powder using grinding machine 10gm of powder was extracted sequentially with ethanol at room temperature for 3 days. Extract were first filtered through Whatman No. 1 filter paper. After filtration the solvent is removed by using Rota evaporator for 2hr. and the extract is collected.

Preparation of Herbal Drops: Concentrated extract is collected then prepare the herbal drops. The concentration of extract in drop is 1mg of extract is dissolved in 1ml of distilled water.

Table 1: Formulation chart of herbal drops

Sr. No.	Ingredient	Quantity
1.	Extracted rose merry leaves	50mg
2.	Distilled Water	50ml

Evaluation

Colour:

The colour of herbal drops was observed visually.

Odour:

The odour of the herbal drops was observed by nose.

Taste:

The tastes of herbal drops are observed by tongue.

pH:

The pH performance of formulated herbal drops the digital pH meter was used to determine and which was calibrated.

The pH measurement has been replicated at three times.

Boiling point:

The boiling point of formulation is measured by using siwoloboff method.

Thin layer chromatography:

The extract was analyzed by thin layer chromatography. In that method the TLC plate is prepared by using silica gel. The thin layer of silica gel is placed on the TLC plate. Dry the TLC plant in hot air oven at temp. 60^0C. The drop extract is applied on the TLC plate with the help of capillary

the TLC plate is placed on the mobile phase chamber. After 90% run of mobile phase on TLC plate it is removed and air dried at room temp.

Stationary Phase:	TLC plate
Mobile Phase :	Toluene : Ethyl Acetate
	95ml :5ml
Coloring Agent:	Not required

Gas chromatography:

The extract were also analyzed by an Agilent-Technologies (Little Falls, California, USA) 6890N Network gas chromatographic (GC) system, equipped with an Agilent Technologies 5975 inert XL Mass selective detector and Agilent-Technologies 7683B series auto-injector. Separation of the extract chemical constituents was carried out on HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm; Little Falls, CA, USA). A 1.0 µL sample volume was injected into the column using the split mode (split ratio 1:100). GC/MS detection was performed by an electron ionization system, with ionization energy of 70 eV. The column oven temperature program was the same as used previously in the GC analysis. The helium was used as carrier gas at a flow rate of 1.5 mL min -1 . Mass scanning range was 50 –550 m/z while the injector and MS transfer line temperatures were set at 220 and 290 ºC, respectively.

In Vitro Anti- microbial Activity:

Method:

Agar Diffusion Assay: Aliquots (5 mg/mL) of the extracts were prepared with the solvent and their antimicrobial activity was further tested against clinical isolates according to the agar diffusion method described. The assay was carried out in Mueller-Hinton agar (Himedia, India). Agar plates were prepared as per the instructions of the manufacturer. Overnight broth culture of the respective test organisms was swabbed on three axes onto the agar with a sterile cotton swab. Then, wells (5 mm in diameter) were made on agar plates by using a sterile cork borer. The resultant wells in triplicate were filled with 100 µL of the plant extract. A well with EtOH was taken as a negative control. Petri plates were then incubated for 24 hrs at 37°C with the exception of S. pyogenes and Campylobacter sp. (inoculated in Mueller-Hinton blood agar and incubated at 37°C in 5% CO2 for 24 hrs). the inhibitory activity was measured by calculating the area of the inhibition zone on three axes. The extent of antimicrobial activities in this study is expressed in terms of area of inhibition zone: 254.34 mm2 , highly active.

Result and discussion

General Evaluation for extract

In order to evaluate herbal extract, the evolution parameters are important tests. It has been established that the pH and taste are satisfactory. The formulation has a pH of 6-7, which corresponds to normal.

Table 2 General evaluation of herbal drops

Sr.No	Characters	Extract
1.	Colour	Green
2.	Odour	Odourless
3.	Taste	Slightly bitter
4.	pH	6.7

Boiling point:

The boiling point it was measured by using siwoloboff method.

Table 3 Boiling point of herbal drops

Sr.No.	Compound	Boiling point
1.	Salvia rosmarinus	170^0C

Thin Layer Chromatography:

The chromatogram obtained from the test solution indicates that a brown area similar in place and colour to the main zone.

The test solution thev chromatogram shows a number of small brown to brownish areas which were less intense than zone corresponding.

Table 4 Rf value of herbal extract

Sr. No.	Chemical Agent	RF value	
		Observed value	Standard value
1.	Rosmarinus officinalis	0.33	0.30
2.	Flavonoids	0.41	0.43
3.	Phenolic acid	0.67	0.68

Gas Chromatography:

From GC-MS analysis, it is clear that the components exist in the sample, which has been eluted from the GC column. Moreover, they are analyzed with an electron impact mass spectroscopy voyager detector. The identification is based on their retention time and mass spectral library. Table 6.4 shows the identified constituents of the herbal extract. The relative amount is calculated based on the peak area. It reveals that the extractl of Rosmarinus officinalis contains a mixture of terpenes that is eluted at different retention times.

Table 5 Retention time of herbal extract compound

Sr. No.	Retention Time	Area [mV.s]	Area [%]
1.	7.124	12.338	1.167
2.	8.864	22.616	2.140
3.	9.228	12.593	1.191
4.	9.824	6.930	0.656
5.	11.264	66.003	6.245
6.	16.62	4.446	0.421
7.	17.068	43.909	4.155
8.	18.068	7.802	0.738
9.	18.788	53.057	5.020
10.	19.616	5.386	0.510
11.	20.268	5.664	0.536
12.	20.416	399.535	37.802
13.	22.168	327.204	30.959
14.	22.376	89.424	8.461

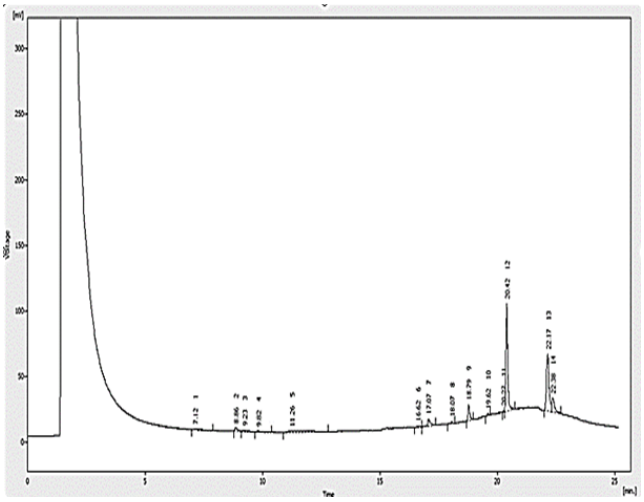

Figure 1 phytochemical screening by gas Chromatography

In Vitro-Anti microbial Activity:

MIC and MBC. MIC, MBC, and MBC/MIC values are presented in Table 6.5. The determined MIC values of R. officinalis correspond to the range of 4. 9^3 to 32. 8^3 µg/ m/L and the concomitant MBC values are registered in the range of 8. 11^3 to 32. 8^3 µg/mL. The lowest MIC and MBC values (4. 9^3 and 8. 11^3 µg/mL) were recorded against Gram-positive isolates, S. aureus. However, the highest MIC and MBC values (16. 9^3 and 32. 8^3 µg/mL) were displayed against Gram-negative isolates, particularly the E. coli. The overall results revealed that antibacterial constituents exist in the extract of R. officinalis. The ratio of MBC/MIC against three meat-borne pathogens was found to be ≤ 4 and therefore the mechanism of antibiosis of plant extract can be inferred as bactericidal.

Table 6 in vitro Anti-microbial activity by agar diffusion method

Organism	R. officinalis		
	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC
S. aureus	4.9^3	8.11^3	2
E-Coli	8.11^3	16.9^3	2

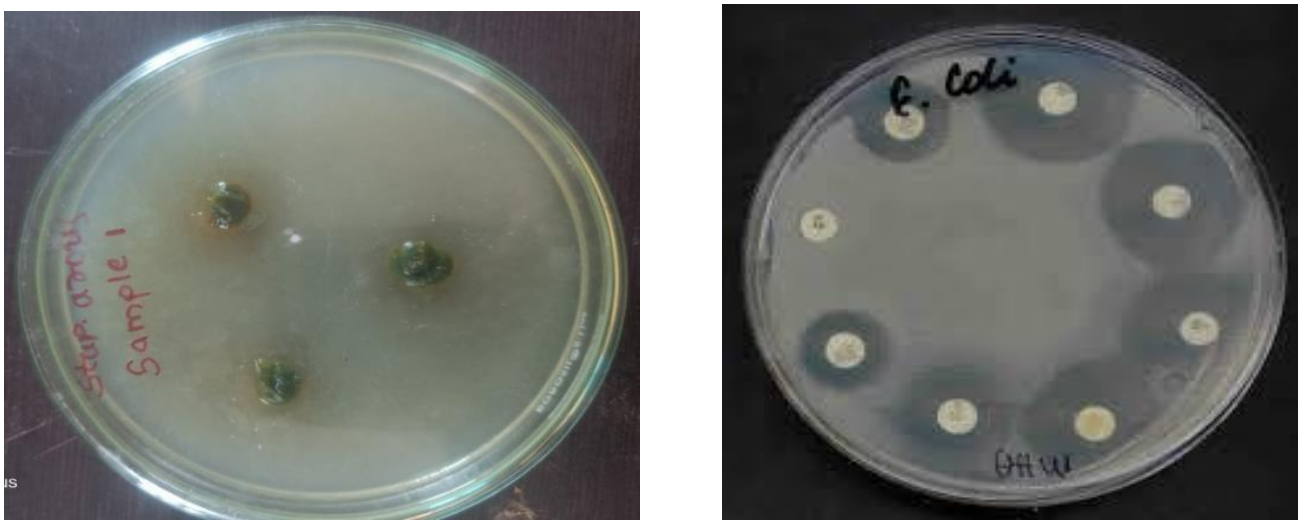

Figure 2 Anti microbial activity of herbal drops against *Staphylococcus aureou* and *E coli*

Conclusion

The aim of this study was to formulate and evaluate Rose merry herbal drops for the formulated herbal drops has shown potential immune booster and anti-diabetic activity and could replace marketed drops with respect to long action. The formulated herbal drop has good comparable anti-microbial activity and anti-diabetic activity as that of marketed formulations. The drops which will be pharmacologically active and has herbal base benefit too. The formulated herbal medicated drops are safe and convenient and easy to use. The phytochemical screening study of herbal medicinal products reveals the presence ofessential chemical constituents in accordance with the standard. The pH of the formulation was found to be 6-7.

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Conflict of interest-The authors declare that they have no conflict of interest.

Informed consent- Not Applicable

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