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Formulation and Evaluation of a Novel Herbal Gel of *Equisetum arvense* Extract

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Equisetum arvense have a great medicinal value for its wound and burn healing properties. Traditionally the plant is used by local people and Ayurvedic physicians mainly for its burn healing properties. The methanolic extract of *Equisetum arvense* was obtained by Soxhlet extraction and then the herbal gel was prepared using the extract in different proportion and different ingredients. The gel was evaluated using the following parameters like appearance, pH, Spreadability, drug release, rheological proerties, extrudability and antioxidant activity useful for effective treatment of wounds and burns. Free radical scavenging activity of a herbal gel prepared from *Equisetum arvense* was measured using DPPH method, Nitric oxide method and H2O2 assay method. The free radical scavenging activity was evaluated for different formulation (EA1-5) and the activity was found in concentration dependent manner

Keyword: *Equisetum arvense,* Horsetail, Hydrogel, Anti-Oxidant Activity.

1. Introduction

Herbal medicine, as a major part of traditional medicine, has been used in medical practice since antiquity and is a common element of ayurvedic, homeopathic, and naturopathic medicine. World health organization (WHO) notes that 74% of the plant derived medicines are used in modern medicine, in a way that their modern application directly correlates with their traditional use as herbal medicines by native cultures[1,2].

Equisetum arvense (Family: Equisetaceae) commonly known as the Field Horsetail or Common Horsetail, is a bushy perennial herb native to the northern hemisphere. It is a member of a very primitive family of plants. In spring a spore-bearing stem, resembling a thin asparagus shoot, rises 15-20cm; once shed, this is replaced by a pale green bush with erect hollow jointed stems with longitudinal furrows, and with sharply toothed sheaths covering each joint; from the sheaths of the central stem arise whorls of fine branches, each giving off finer whorls, the whole sometimes extending up to 60cm in height[3,4].Active Compounds of the plant include minerals like silicic acids and silicates, potassium, sulphur, manganese, magnesium; flavonoids: quercetin glycosides; phenolic acids, alkaloids, equisetonin, phytosterols: cholesterol, isofucosterol, campesterol; tannins.[5,6] Horsetail possesses diuretic properties, which are believed to be due to equisetonin and flavone glycosides[7]. Horsetail herb extract helps body retain calcium more efficiently due to a silica compound and can

even help repair bones and cartilage. This is certainly essential for managing joint degeneration conditions or hard to heal bone fractures. Osteoporosis is one among many diseases that horsetail extract benefits [8]. Horsetail is known for its anti-inflammatory, anti-nociceptive [9], antioxidant and anti-proliferative [10], antimicrobial [11-13], hepatoprotective [14], anti-diabetic [15], coagulant and astringent activity[16].

A hydrogel is a three-dimensional network of hydrophilic polymer chains that could be cross-linked through either chemical or physical bonding. Because of the hydrophilic nature of polymer chains, hydrogels are capable of swelling when placed in aqueous media, *i.e.*, they retain a significant amount of water but remain water-insoluble. When the polymers are cross-linked, the hydrophobicity of a gel is increased and the diffusion rate of the drug is diminished. These characteristics of hydrogels, as well as their biocompatibility, increased duration of action with increased therapeutical efficiency due to the viscosity of the gel matrix and soft consistency (easy and safe administration at home by nonmedical persons)[17,18].

Topical application of gels at pathological sites offer great advantage in a faster release of drug directly to site of action, independent of water solubility of the drug as compared to creams and ointments[19,20].

2. Materials and Methods

The required quality of Carbopol-934 was slowly sprinkled into purified water I.P with constant stirring to get a uniform dispersion and then kept overnight for hydration. The accurately weighted amounts of drug along with other additives were poured into the fixed amount of hydrated Carbopol-934 dispersion with constant stirring. Finally the required amount of 0.5M sodium hydroxide solution was added to induce gelation. The composition of the herbal gel prepared from methanolic extract of *Equisetum arvense* is tabulated in Table 1.

Fig 1: *Equisetum arvense* plant

2.1 Preparation of Gel Containing Extract

Fixed proportions of Carbopol 934 and Sodium CMC was dispersed in 50 mL of distilled water with continuous stirring. 5 mL of distilled water was taken and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath. Cool the solution, then to that added glycerin and mixed it with first solution. Further required quantity of *Equisetum arvense* plant extract was mixed to the above mixture and volume made upto 100 mL by adding remaining distilled water. Finally full mixed ingredients were mixed properly to the Carbopol 934 gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency. The same method was followed for preparation of control sample without adding any *Equisetum arvense* plant extract.

2.2 Evaluation of gel

The above formulated gel formulation was subjected to evaluation of following parameters[21].

2.3 Physical observation

Physical parameters such as color, appearance and feeling on application were recorded. All

formulations were observed visually for their clarity and color.

2.4 Homogeneity

The homogeneity of all developed gels was checked visually for the presence of any aggregates or clumps and for appearance.

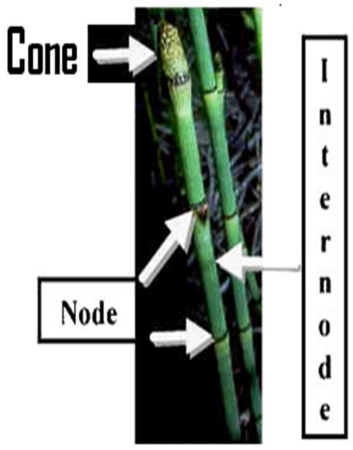


Fig 2: ***E.arvense*** **showing node, internodes and cone**

2.5 Determination of pH: Accurately weighed 5 gm of gel was dispersed in 45ml of water to determine the pH of the suspension using digital in pH meter. The determinations were carried out in triplicate and the averages of three readings were noted.

2.6 Viscosity: Viscosity of the gel was measured using Brookfield viscometer with spindle type 4 with 30 gear speed.

2.7 Determination of Spreadability:

It was determined by parallel plate method. Two glass slide of 10 x 20cm were selected. The gel formulation whose spreadability had to be determined were placed over one slide. The other slide was placed upon the top of the formulation such that the gel was sandwiched between the two slides across a length of 14.5 centimeters along the slide. Two slides are fixed to stand so that lower slide was remained fixed allowing the upper slide to slip off freely with the help of 50 gm weight.

The time required for the upper slide to separate out from lower slide was noted and spreadability was calculated as follows.

$$S=W \times L/T$$

Where,
S=Spreadability, L=length of the glass plate (14.5 cm),
W=Weight tied to upper plate (50gm),
T=Time taken to separate the slide completely from each other.

2.8 Extrudability

The formulations were filled into collapsible aluminium tubes and sealed by crimping machine. The weight of tubes was recorded. The tubes were placed between two glass slides weight500gm was placed on the slide and then cap was removed, the amount of extruded gel was concluded) after the gels were set in the container. The extrudability of formulation was determined as if the extrudability is >90% then it is excellent; >80% extrudability then it is good; >70 extrudability then it is fair.

2.9 Drug Content

A specific quantity of gel, 200mg is extracted in 50ml of ethanol for 1 hr. This solution was then filtered, residue washed twice with ethanol and then volume produced to give 100 ml solution in volumetric flask. The drug content in this solution was then estimated spectrophotometrically at 269nm using ethanol as blank using calibrated curve.

2.10 Stability study All the selected formulations were subjected to a stability testing at different condition (at 30°C ± 2°C/65% RH ± 5% RH and 45°C ± 2°C/75% RH ± 5% RH) for three months as per ICH norms. All selected formulations were analyzed for the change in appearance, pH and drug content as per ICH norm[22].

Table 1: Formulation of topical gel of *Equisetum arvense* plant extract

Ingredients (% w/w)	**Formulations**				
	EA-1	EA-2	EA-3	EA-4	EA-5
Equisetum arvense extract	1	2	3	4	5
Carbopol 934	2	2	2	2	2
Sodium CMC	1	1	1	1	1
Glycerin (mL)	2	2	2	2	2
Methyl Paraben (0.5%)(mL)	0.15	0.15	0.15	0.15	0.15
Propyl Paraben (0.2%)(mL)	0.5	0.5	0.5	0.5	0.5
Triethanolamine	qs	qs	qs	qs	qs
Water qs	100ml	100ml	100ml	100ml	100ml

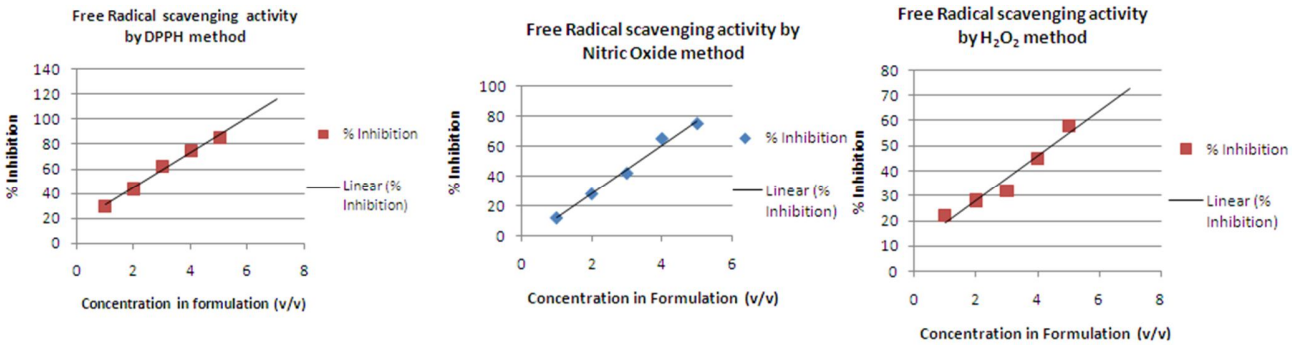
Table 2: Physical evaluation of topical gel of *Equisetum arvense* plant extract

Formulation	**Color**	**Appearance**	**Spreadibility (g.cm/sec)**	**Viscosity (cps)**	**pH**	**Homogeneity**	**Extrudability**
EA1	Yellow	Homogeneous	16.65	1550	6.7	Very Good	Excellent
EA2	Yellow	Homogeneous	15.25	1540	6.8	Very Good	Excellent
EA3	Yellow	Homogeneous	14.00	1650	6.8	Good	Excellent
EA4	Yellow	Homogeneous	13.46	1490	6.7	Very Good	Good
EA5	Yellow	Homogeneous	12.55	1850	7.4	Good	Good

Table 3: Stability testing at 3rd months of topical gel of *Equisetum arvense* plant extract

Formulation	Temp.	Appearance	Spreadibility (g.cm/sec)	pH	Drug Content	Extrudability
EA1	25	Homogeneous	15.20	6.6	98.55	Excellent
	30	Homogeneous	16.55	6.6	98.40	Excellent
	40	Homogeneous	15.65	6.7	96.20	Excellent
EA2	25	Homogeneous	15.50	6.5	98.55	Excellent
	30	Homogeneous	16.30	6.6	99.40	Excellent
	40	Homogeneous	15.60	6.4	98.80	Excellent
EA3	25	Homogeneous	15.40	6.8	97.40	Excellent
	30	Homogeneous	16.80	6.6	98.80	Excellent
	40	Homogeneous	15.30	6.6	98.50	Excellent
EA4	25	Homogeneous	15.50	6.7	98.55	Excellent
	30	Homogeneous	16.20	6.5	98.40	Excellent
	40	Homogeneous	15.60	6.6	98.90	Excellent
EA5	25	Homogeneous	16.40	6.4	98.55	Excellent
	30	Homogeneous	15.85	6.6	99.40	Excellent
	40	Homogeneous	15.70	6.6	98.55	Excellent

Graph 1. Concentration dependent antioxidant activity of topical gel of *Equisetum arvense* by DPPH, NO and H2O2 assay method



2.11 Nitric oxide scavenging activity:

A potential determination of oxidative damage is the oxidation of tyrosine residue of protein, peroxidation of lipids, and degradation of DNA and oligonucleosomal fragments. Sodium nitropruside in aqueous solution at physiologic pH (7.4) spontaneously generates nitric oxide, which interact with oxygen to produce nitrite ions, which can be determined by use of Griess reagent. The method of Akiri et al [24]was adopted to determine the nitric oxide radical scavenging activity of O/W sunscreen cream. Two milliliter of 10 mM sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of the equally diluted sample of formulation (1–5 % v/v). The standard sample was processed in same manner and all the mixture were incubated at 25°C. After 150 min, 0.5 ml of incubated solution was withdrawn and mixed with 0.5 ml of Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture was subjected for incubation at room temperature for 30 min. The absorbance was measured at 540 nm. The amount of nitric oxide radical was calculated by following this equation:

% inhibition of NO = [Abs (control) –Abs (standard) / Abs (control)] × 100

2.12 Scavenging of Hydrogen peroxide:

The ability of the formulations to scavenge hydrogen peroxide was estimated according to the method of Bhuiyan *et al*[25]**.** A solution of hydrogen peroxide (25mmol/l) was prepared in phosphate buffer (pH 7.4). The sample of diluted formulation (1–5 % v/v) and diluted standard cream sample was added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was taken after 10 min against a blank solution having phosphate buffer in absence of hydrogen peroxide. For each concentration, a separate blank sample was used. The percentage scavenging activity of hydrogen peroxide by sample and standard formulations were calculated using the following formula,

% scavenging activity [H2O2] = [Abs (control) – Abs (standard) / Abs (control)] × 100.

3. Results and Discussion:

The various physicochemical properties of the prepared gel formulations are shown in Table 2. From the results it is clearly evident that all the gel formulations showed good gelling property and homogeneity. The pH of all the formulations was in the range of 6.77 to 7.28, which lies in the normal pH range of the skin. Viscosity is the most important parameter in the evaluation as it governs the many properties of the formulation such as, spreadability, pourability of the product from the container etc. Viscosity for respective gel was found to be 1550, 1540, 1650, 1490 and 1850 cps at 30 gear speed (Table 2). The values of spreadability indicate that the gel is easily spreadable by small amount of shear. The results of spreadability were found to 16.65, 15.25, 14.00, 13.46 and 12.55 gm.cm/sec. Since packing of have gained considerable importance in delivery of desired quantity of formulation from the container, the measurement of extrudability becomes an important criteria. All Gel formulations had an excellent extrudability with>90% extrudability (Table 2). The stability test (Table 3) was carried out for three months and results revealed that the all gels showed better stability. During stability study, there was not much variation in viscosity after testing at different temperature conditions. Extrudability and spreadability were also measured and found to be less variant than the initially prepared gel after performing stability study. There was no significant change in pH values. The drug content was in the range of 96.20 to 99.40 %.

Several concentrations ranging from 1-5% of *Equisetum arvense* methanol extract were tested for their antioxidant activity using different *invitro* models. It was observed that free radicals were scavenged by the test compounds in a dose dependent manner in the various methods. Dose-response curve of DPPH radical scavenging assay, H_2O_2 assay and NO assay method of the all the samples are presented in Graph 1. By the DPPH assay method, different Formulation (EA 1-5) showed 32, 46, 62, 78 and 88% antioxidant activity respectively.

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