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Research Article

FORMULATION AND EVALUATION OF CREAM USING *Moringa oleifera* L. LEAF EXTRACT

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ABSTRACT

There are an increasing number of studies on the cosmeceutical properties of medicinal plants growing in various climatic zones. The formulation of phenolic rich plant extracts into cosmetic cream products can maximize the commercial value of such products. Most of the herbs used in Nepal for cosmetic purposes are locally available Ayurvedic herbs. The antioxidant property of the ethanolic extracts of *Moringa oleifera* L. leaves collected from Rupandehi and Makawanpur districts respectively were determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. Total phenolic and total tannin content was determined by Foline Ciocalteu reagent. Total phenolic content was found from 35.51 and 42.89 mg/100 g gallic acid in the Makawanpur and Rupandehi districts. Overall, findings revealed that there were slight differences in antioxidant properties between the two samples. The cream formulated from the Makawanpur plant sample had considerable physiochemical parameters within the range of acceptance. These findings provide strong evidence for further development of commercial creams with antioxidant properties.

Keywords: Antioxidant, cream, *Moringa oleifera* L.*,* phytochemicals, total phenolics

INTRODUCTION

Moringa oleifera L. is the most widely cultivated of the 13 species of family Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh, Afghanistan, and Nepal, which is now indigenous to many regions Africa, Southeast Asia, and South America (Fahey, 2017). A plethora of studies that have been published in the decade describe the popularity of moringa as a miracle tree. It is well known plant for nutritional as well as potential antioxidant (Bharali *et al.,* 2003), anticancer, anti-inflammatory, antidiabetic, antimicrobial agent and possesses numerous medicinal qualities (Mahmood *et al.,* 2010). Several valuable reviews of the ethnobotanical uses of *M. oleifera* L are available with a variety of potential uses (Hussain *et al*., 2014, Aney *et al.,* 2009). The leaves have been established as a rich source of phenolics and glucosinolates (Sreelatha & Padma, 2009; Amaglo *et al.,* 2010), carotenoids (Saini *et al.,* 2014), isothiocyanates (Waterman *et al.,* 2014) and various protein, vitamins, amino acids and phenolics (Anwar *et al.,* 2007). It has also long been labeled for its great cosmetic value in which in recent years, commonly found to be used in various health care products such as moisturizers and conditioners. Moisturizers containing herbal antioxidants are active for dry skin, daily safeguarding of normal skin, and adjunctive therapy related to many skin diseases (Dal'Belo *et al.,* 2006). Phenolic antioxidants inhibit inflammation, which leads to collagen improvement, and may offer protection against skin photo-damage. Cream containing leaf extract is a natural effective ingredient for improving hydration, which can be used in moisturizing cosmetic formulations and also to complement the treatment of dry skin (Sahu *et al*., 2011; Ali *et al.,* 2013; Gyawali & Paudel, 2022). Skin care herbal products that claim therapeutic benefit from addition of plant extracts and active ingredients such as α-hydroxy acid, retinoic acid, ascorbic acid and coenzyme Q10 (Gyawali *et al.,* 2020; Gyawali *et al.,* 2016; Kumar *et al.,* 2016; Knott *et al*., 2015).

To promote the use of Nepalese medicinal plants as potential sources of skin care products, it is important to thoroughly find out their phytochemical profile, bioactive properties, and design a suitable formulation. Undertaking these precedents into consideration, the aim of this study was to evaluate the phytochemical constituents and antioxidant activity of ethanolic extracts of *M. oleifera* and then develop a functional cream.

MATERIALS AND METHODS

Plant Material

M. oleifera L leaves were collected from Makawanpur and Rupandehi district of Nepal during April 2018. The plant material was identified by lead author, and crude drug specimen were deposited at the Department of Pharmacy, Kathmandu University, Nepal.

Preparation of Plant Extracts

The leaves were sun dried, grinded into powder, and passed through 80 size sieving trays. Extraction was carried out in ethanol by using Soxhlet apparatus for continuous 8 hours at temperature and then evaporated by using rotavapor (Buchi R-215, Switzerland), 75-90 RPM, under 100 mbar pressure maintaining 40°C temperature of water bath.

Phytochemical screening

Phytochemical screening for the class of carbohydrate, tannin, phenolics, flavonoids, glycosides and terpenoids was carried out by using a previously described standard method (Trease & Evans, 2002).

Thin layer chromatography

Thin layer chromatography (TLC) was performed on readymade Silica gel TLC plates. The plates were dried in hot air oven at 110°C for 30 minutes, stored in a dry atmosphere and then used for analysis. Samples of all fractions were prepared by diluting the crude extracts and then applied usually 10*µ*L volumes to the origins of a TLC plate 2 cm above its bottom with the help of capillary tubes and run into a suitable mobile phase until the separation of spots. Chloroform, Methanol and Water in the ratio of 7:3:0.5 ratio was optimized as a best mobile phase for the separation of wide range of compounds.

Antioxidant activity

Antioxidant activity was determined by a previously described method (Brand-Williams *et al.,* 1995) with slight modification. All samples were calculated in triplicate. Stock solution of 100µM of DPPH and the extract was prepared in 10, 20, 30, 40 and 50*µ*g/mL concentrations in methanol. Similarly, reference samples of ascorbic acid were made at similar concentrations (Fig. 1). A total 2.0 mL of 100*µ*M DPPH was added to 2.0 mL of each extract of different concentration and kept in dark. Similarly, 2.0 mL of 100*µ*M DPPH was mixed with 2.0 mL of methanol and ascorbic acid and kept in dark for 30 minutes in incubator at 37°C. The absorbance was measured at 517 nm by UV spectrophotometer after 30 minutes and percentage scavenging was calculated by using the following equation:

% scavenging = $(A_0\text{-}A1) / A_0 X\ 100$ %
Where, A_0= Absorbance of control
A1 = Absorbance of test samples

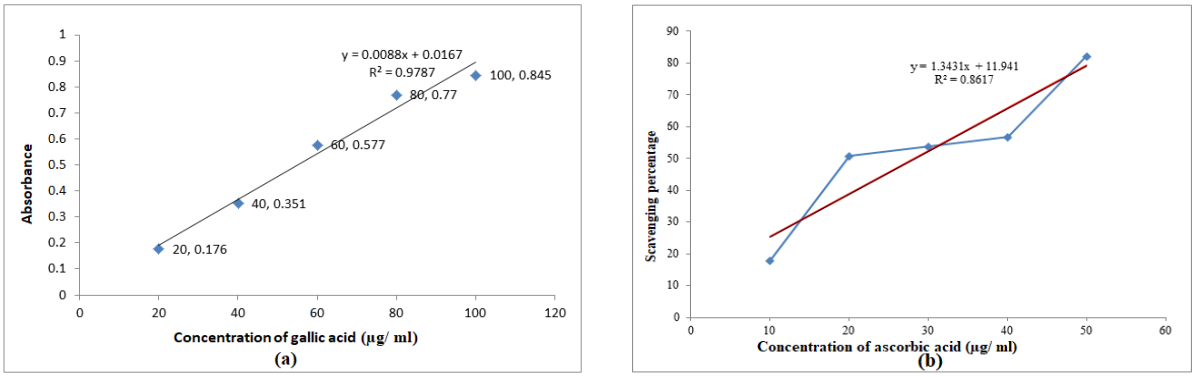

Figure 1. Calibration curve of (a) gallic acid and (b) DPPH scavenging activity of ascorbic acid in the determination of antioxidant activity of *M. oleifera* L.

Determination of total phenolic content

The total phenolic content of each extract sample was evaluated using the Foline Ciocalteu reagent described previously (Singleton *et al.,* 1999), with slight modifications. Each extract sample was dissolved in deionized water until 100 *µ*g/mL. The standard curve was obtained using gallic acid (0, 20, 40, 60, 80, and 100 *µ*g/mL). Each diluted extract sample or gallic acid (2.0 mL) was added to reagent (0.5 mL, 20 %) and mixed well for 5 minutes. Sodium carbonate (0.5 mL, 10 %) was added to each mixture and all the mixtures were kept at room temperature for 1 h. The absorbance of all the mixture was measured at 765 nm using a UV-visible spectrophotometer.

Determination of total tannins content

The total tannins were determined by Folin-Ciocalteu method (Sultana *et al.,* 2012) with slight modification. About 0.1 mL of the sample extract was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu phenol reagent, 1 mL of 35% sodium carbonate solution and dilute to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. a set of reference standard solutions of gallic acid (0, 20, 40, 60, 80 and 100 *µ*g/ mL) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with a UV/ Visible spectrophotometer. The estimation of the tannin content

was carried out in triplicate. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample.

Determination of ash value

About 3 g of each of the crude powders were accurately weighed and taken separately in a silica crucible, which was previously weighed. The powder was incinerated in a Muffle furnace at 450°C. The remaining inorganic material was cooled in desiccator, weighed and total ash content was determined. Water soluble ash was determined by boiling ash in water for 5 minutes and calculating the insoluble fraction. The sulphated ash was determined by digesting the ash with sulphuric acid (IP, 1985; Lachman *et al.,* 1999).

Cream formulation

For the preparation of cream, based on the primary result of antioxidant property, the sample from Rupendehi district was taken. The oil phase was taken in the beaker and warmed. Then aqueous phase and the water-soluble excipients were also warmed. Then both materials were mixed to prepare cream. The excipients were gradually added to the mixture and thoroughly mixed in mixture. The batches were evaluated for parameters like viscosity, pH, washability, softness, appearance, irritancy and spreadibility.

Table 1. Composition of *M. oleifera* L extract cream

Water phase (%)		Oil phase (%)	
Glycerin	3.00	Stearic acid	6.00
Titanium dioxide	0.30	Cetyl alcohol	4.00
Extract	o.10	Methyl paraben	0.30
Methyl paraben	0.10	Propyl paraben	0.30
Propyl paraben	0.10	Glyceryl mono sterate	4.00
Water	qs	Petroleum jelly	3.00

RESULTS AND DISCUSSION

Phytochemical Screening

Preliminary phytochemical screening showed that both samples contain tannin, phenolics, flavonoids, glycosides and terpenoids as a major class of secondary metabolites. Similar type of results was also obtained in previous findings (Amaglo *et al.,* 2010; Saini *et al.,* 2014). The leaves, in particular, have been found to contain phenolics and flavonoids (Anwar *et al.,* 2007; Verma *et al.,* 2009).

Thin Layer Chromatography

TLC profiling of ethanol extract indicated the separate spots in normal visible light. The separated compounds were observed while exposed in the UV at short wavelength after 10% H_2SO_4 spraying. It shows that in both TLC of extracts, a similar pattern of compounds was detected, however Rupendehi sample was lacking one spot at normal visible light (Fig. 2).

Antioxidant Activity

The antioxidant activity of the ethanolic extracts was determined by DPPH method and the results are presented in Table 1. Our experimental results showed that the average antioxidant activity was found in the as compared to the standard ascorbic acid (IC_{50} with 28.33 µg/mL). The free radical scavenging effect of leaf extract of both the samples was comparable with that of the reference antioxidants. The result suggests that both samples have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage as in previous study (Sreelatha & Padma, 2009). The present study revealed that extracts exhibit radical scavenging activity probably derived from phenolic phytochemicals reacting uniquely with various free radicals. As we have extracted our samples in the ethanol, it agrees with the previous studies which have suggested that extracts obtained from polar solvents were likely to show higher antioxidant activity (Siddhuraju *et. al.,* 2003; Verma *et al.*, 2009).

Total Phenolic and Total Tannin Content

Flavonoids are probably the most important class of natural phenolics of *M. oleifera* L., so they can directly scavenge reactive oxygen species (Siddhuraju *et al.,* 2003). Total phenolic content was found from 35.51to 42.89 mg/100 g gallic acid, which is slightly different from the previous finding of total phenolic compounds in the leaf of *M. oleifera* L. 170.07 ± 0.43 mg /100 g gallic acid (Nascimento *et al.,* 2017). We found that the *M. oleifera* L. have high levels of flavonoids and low levels of tannins. Similar difference on concentration of total phenolics and tannins was also observed in previous study in *M. oleifera* L. (Mohammed & Manan, 2015).

Determination of ash value

The Makawanpur sample gave higher ash content of 21.55% whereas Rupendehi sample gave less ash content of 15.88% which may be due to presence of high inorganic content. However, the ash content is possibly due to the

Na^+ and Ca^{2+} salts which are not harmful. Thus, ash content value also reflects plant was high in minerals and vitamins. In the recent study, total ash was found 15.88 and 21.55%, which is also in agreement with previous finding 9.15% (Mahima *et al.,* 2014) and in another study, 14.93% (Mikore & Mulugeta , 2017) of same plant.

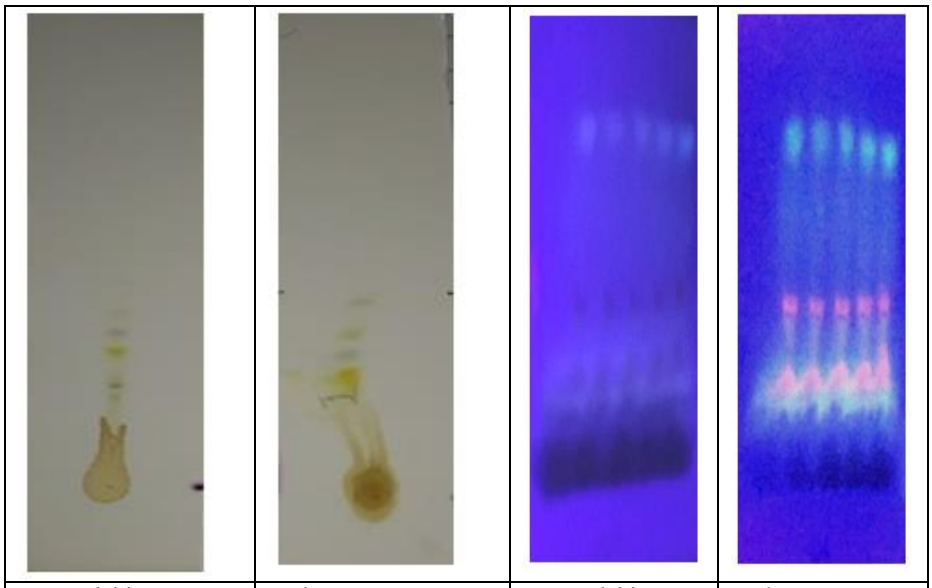

Figure 2. TLC of *M. oleifera* L leaf extracts collected from Makawanpur and Rupendehi districts.

Table 2. DPPH scavenging activity of ethanolic extract of *M. oleifera* L. leaf collected from Makawanpur and Rupendehi districts.

Concentration (µg/ mL)	Scavenging percentage of Makawanpur sample	IC 50 (µg/mL)	Scavenging Percentage of Rupendehi sample	IC 50 (µg/ mL)
10	12.08	30.64	10.59	32.03
20	25.52		23.43	
30	43.58		33.88	
40	71.79		70.00	
50	90.44		91.04	

Table 3. Total phenolic content and total tannin content of ethanolic extract of *M. oleifera* L

Plant source	Yield percentage	Total phenolic content (mg/100g gallic acid/g)	Tannin (mg/100g gallic acid/g)
Makawanpur	6.83	35.51	0.94
Rupendehi	6.73	42.89	0.75

Table 4. Total ash, water soluble ash and sulphated ash content of ethanolic extract of *M. oleifera* L

Plant source	Total ash	Water soluble ash	Sulphated ash
Makawanpur	21.55	0.136	30.1
Rupandhei	15.88	0.106	32.2

Evaluation of herbal cream

The pH and viscosity of the creams were found to be in the range. The creams applied on the skin were easily removed by washing with tap water and finally the formulations showed no redness, inflammation, and irritation during irritancy studies. The seed oil cream of *M.*

oleifera L. was also prepared and evaluated by previous researchers and found a significant 70% reduction in paw edema was observed (Suryadevara *et al*., 2018). Our cream consists of 0.1% extract with satisfactory physiochemical properties, however in one previous study, it was shown that the concentration of *M. oleifera* L. leaf extract can be used as anti-aging in which the decreasing evenness was 3% in the cream formulation (Sugihartini & Nuryanti, 2017).

Table 5. Physiochemical properties of cream prepared of *M. oleifera* L. extract

Parameters	Property
pH	The pH of the creams was found to be in the range of 5.6–7.0 which is good for skin.
Viscosity	The viscosity of creams was in the range of 2.6–3.7 cps which indicates that the cream is easily spreadable by small amounts of shear.
Homogeneity	The formulation produced a uniform distribution of extracts in cream. This was confirmed by visual appearance and by touch.
Appearance	When formulation was kept for three months, it was found that there was no change in color of cream.
Softness	Slipperiness, and amount of residue left after the application of the fixed amount of cream were found.
Washability	The creams applied on the skin were easily removed by washing with tap water and showed a good washability.
Irritancy	The cream showed no redness, inflammation, or irritation during irritancy studies. These formulations are safe to use for skin.

CONCLUSIONS

M. oleifera L. leaves from Makawanpur and Rupandehi was found rich in phytochemicals phenolics with good antioxidant property with IC_{50} values of 30.64 and 32.03 µg/mL. The cream formulated by adding leaf extract exhibited considerable physiochemical parameters within the range of acceptance. The result shows the baseline evidence for the further development of commercial cream of antioxidant property.

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AUTHORS CONTRIBUTION STATEMENT

RG: Project concept, supervision, paper writing, and editing; DB, NB, RB, SB, and SS: laboratory work and draft preparation; PNP: paper writing and final editing.

CONFLICT OF INTEREST

The authors do not have any conflict of interest pertinent to this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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