

COMPARATIVE ANALYSIS OF ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF TRANSDERMAL PREPARATIONS CONTAINING CASTOR OIL

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ABSTRACT

Background: Medicinal plants play vital role in preservation of healthy human life. In these medicinal plants *Ricinus communis* has great importance that belongs to the family Euphorbiaceae. It is found all over Pakistan. Castor Oil obtained from this plant is highly useful in Homeopathic, Ayurvedic, and Unani system of medicines as a fertilizer, fungicidal, antibacterial, cathartic and purgative. It also used as a labor pain inducer and as an ingredient in many cosmetics products for preventing wrinkles, fight acne, as a moisturizing ingredient, soothing the sunburn and reduce inflammation of skin. **Method:** Two types of transdermal patches were formulated, one containing combination of castor oil and acetone emulsion A while other formulation emulsion B was contain castor oil, acetone and antioxidants i.e. Vitamin C and E. Both formulations were evaluated by in vitro for antibacterial and antioxidant activities. **Results:** The outcomes indicated that transdermal patch containing emulsions A have TPC 136, TFC 59.8, reducing power assay 74%, DPPH 75% of inhibition. Transdermal patch containing emulsion B have TPC 150, TFC 77.23, reducing power assay 51%, DPPH 61.90% of inhibition in vitro antibacterial activity showed that castor oil + acetone emulsion containing patches showed 4 µg/ml MIC for *E. coli* and 2 µg/ml MIC for *S. Aureus*. For, vivo testing, these transdermal patches was applied on skin wounds of rabbits to study its wound healing activity. **Conclusion:** It was concluded that transdermal patch containing emulsions B have more efficacies in wound healing then patches A at the end of 20 days.

Key words: Medicinal plant, *Ricinus communis*, Transdermal patch

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INTRODUCTION

Transdermal patches are widely used as topical, cosmetic and transdermal drug delivery systems. These patches represent a key outcome in growth in skin science, technology, clinical observation and evidence-based studies that date back to the first existing human records. The skin is the largest organ in the human body by mass. Drugs have been applied to the skin to treat superficial disorders like fungal infections dermatitis, other injuries and for the transdermal administration of therapeutics to manage systemic level of drug and as cosmetics to reduce wrinkles and dark spots. For instance, the use of ointments and patches, consisting of plant, animal or mineral extracts, was already very popular in ancient Egypt and in Babylonian medicine (around 3000 BC) [1]. The routine use of transdermal delivery systems became a common practice in the latter third of the 20th century when delivery technology was developed to enable reproducible administration through the skin for systemic effects of drug.

The goal of this research is to detail the rich history of topical and transdermal delivery that has evolved over thousands of years, focusing particularly on the

evolution and current use of transdermal patches on the skin of humans. The potential efficacy and suitability of this technology for systemic therapy is normally determined by drug blood level–time profiles, which can be compared to or predicted from per oral or parenteral administration of this drug because parenteral administration have 100% bioavailability [2]. These drug concentrations in the blood are defined by the amount of drug released into the body from the delivery system and the application area. Transdermal delivery is also used to produce clinical effects such as anti-inflammatory activity, local anesthesia and deep within or beneath the skin. In contrast, topical delivery seeks to treat superficial, although at times very serious, skin problems to produce a relatively local action [1].

Plant kingdom provides good health to human beings and animals. These are the rich sources of organic compounds and medicinal constituents. Many natural crude drugs are available that are pharmacologically active against many diseases and disorders. In these crude drugs one of them is *R. communis*, which is also known as "castor plant" and commonly known

by different names like palm of Christ, Verenda (Bengali), Jada (Oriya), Errandi (Marathi). The plant mostly grows on tropical regions. The stem of *Ricinus communis* has antidiabetic, antiprotozoal and anticancer activity [3]. The root, leaf and seed of castor oil plant have been used for the treatment of the inflammation, liver diseases, hypoglycemic, laxative and purgative [4].

The studies of castor oil prove the presence of palmitic, stearic, arachidic, hexadecenoic, oleic, linoleic, ricinoleic and dihydroxy stearic acid. The stem of castor oil plant contains ricinolic acid [3]. Castor oil has many benefits like it has antibacterial activity so can be use in bacterial skin infections i.e. acne. Castor oil also contains natural antioxidant ricinolategallic acid that can fight the free radicals and reduce aging process and wrinkles. It has anti inflammatory properties thus reduced the inflammation of tissue or acne and around the eyes. It relieves the pain associated with sunburn and reduces peeling. It can keep the skin smooth shiny, acne and wrinkle free. Castor oil is also an important ingredient of lipsticks and lip gloss to smooth the skin of lips. Castor oil contain high amount of free fatty acids that are very essential to maintain good health of skin [5].

R. communis has activity against Streptococcus species, Klebsiella pneumonia, Candida albicans, Proteus vulgaris, Staphylococcus aureus and Escherichia coli. Petroleum ether and acetone extracts have excellent zone of inhibition with castor oil but ethanolic extract shows anti-bacterial activity only at high concentration. Immunocompromized patients suffering with oral cancer develop secondary infections due to different bacterial and fungus species. These infections are treated with co-administration of *Ricinus communis* with the immunosuppressant drugs [6]. Similarly vitamin C and vitamin E have antioxidant properties and also has activity in biosynthesis of collagen pathways. So we are proposing the transdermal preparation containing Castor oil and acetone emulsion along with vitamin C and E as an antioxidant to check their antibacterial and antioxidant properties in vitro and wound healing properties in rabbit skin.

Currently, no castor oil transdermal patches are available commercially along with acetone and antioxidants. So, the present study is designed with the following objectives: 1. To prepare the transdermal patches containing castor oil, acetone and vitamin C & E. 2. To check the in vitro antibacterial/antioxidant activity of transdermal formulations. 3. Comparative analysis of wound healing properties of both transdermal formulations in rabbits.

MATERIAL AND METHOD

Preparation of Castor Oil and Acetone Emulsion

Pestle and motor was used to triturate the castor oil and gum acacia (emulsifying agent). Then acetone was added to distilled water to make 70% aqueous concentration of acetone. This was solution B. this solution was added drop wise into castor oil and gum acacia in pestle and motor and vigorously triturate it until homogeneous mixture was formed. This emulsion was ready to load into transdermal patches.

Table 1: Ingredients composition for emulsion A.

Ingredients	Official formula	Formula for 100ml
Castor oil	375ml	3.75ml
Acacia	100 gram	10 gram
Acetone	700 ml	70ml
Distilled water	1000ml	100ml

Preparation of Castor Oil, Acetone and Vitamin C and E Emulsion

Clean pestle and motor and was used to triturate the castor oil and gum acacia (emulsifying agent). Then vitamin E and vitamin C was added to castor oil and gum acacia and triturated vigorously. Then acetone was added to distilled water to make 70% aqueous concentration of acetone. This was solution B. then solution B was added drop wise into pestle and motor and vigorously triturates it until homogeneous mixture was formed. This was poured into plastic container.

Table 2: Ingredients composition for emulsion B.

Ingredients	Official formula	Formula for 100ml
Castor oil	375ml	3.75ml
Acacia	100 gram	10 gram
Acetone	700 ml	70ml
Distilled water	1000ml	100ml
Vitamin C	10% w/w	10 gram
Vitamin E	5% w/w	5 gram

In-vitro Anti Bacterial Activity of Both Formulations

In vitro antibacterial activity of castor oil and acetone formulation and castor oil, acetone and vitamin C and E formulation was evaluated against two bacterial strains i.e. one Gram positive (*S. aureus*) and Gram negative strain (*E. coli*). Results of antibacterial activity were calculated in term of Minimum Inhibitory Concentration (MIC) by Micro dilution test method. To check MIC microdultion method was performed. 10% aqueous dilutions of prepared patches were prepared in a 96-well micro titration plate. 10µl of indicator solution and 10µl of Muller

Hinton broth were added to each well of plate. After this, 50 µl of bacterial suspension was also added to each well plate prepared in triplicates and placed in an incubator at 37 °C for 18 to 24 hours. After that change in color was evaluated visually. MIC value was taken at lowest concentration at which visible color change was evaluated. The growth of an organism was indicated by turbidity of solution.

In-vitro Antioxidant Activity of Both Formulations

Antioxidant activity of castor oil and acetone formulation and castor oil, acetone and vitamin C and E formulation was tested in vitro by total flavonoid contents (TFC), total phenolic contents (TPC), DPPH (1, 1-diphenyl-2-picrylhydrazyl) reducing power and scavenging activity following the method of [7].

Determination of Total Phenolic Contents (TPC)

Total phenolic contents of preparation were determined by Folin-Ciocalten method. Amount of total phenolic contents were assessed using the Folin-Ciocalten reagent. For calibration curve, 1ml aliquots of 50, 100, 150, 200, 250 and 300 mg/ml gallic acid solution in ethanol was mixed with 5ml of this reagent and 4ml of sodium carbonate solution (20%). After incubation of 1 hour at room temperature, measure the absorbance of resulting blue color at 765nm and calibration curve was plotted. The result was expressed as gallic acid equivalents g/100g of dry plant matter. TPC was expressed from linear regression curve of gallic acid.

Determination of Total Flavonoid Contents (TFC)

The TFC were measured by spectrophotometric method. The emulsion was mixed with water in 10ml of volumetric flask. Initially 5% NaNO₂ solution was added to each flask after 5 minutes 10% AlCl₃ was added and 6 minutes was added. Water was added to flask and mix well. Then check the absorbance at 510nm after incubation for 15 minutes. Total flavonoid contents were expressed as catechin equivalents from linear regression curve of catechin.

Determination of Reducing Power

The reducing power of extract of *R. communis* was determined by mixing with sodium phosphate buffer (5, 0.2 M, pH 6.6) and potassium ferricyanide (K₃Fe(CN)₆) (5ml, 1%) this mixture was incubated for 20 minutes at 50 °C. trichloroacetic acid was added to this mixture and centrifuge it for 10 minutes at 5°C at 980g. The upper layer was diluted with 5 ml of distilled water and ferric chloride and read the absorbance at 700nm by using spectrophotometer. Increase absorption of reaction indicates increased reducing power. The % reducing power of samples was calculated by following formula:

$$\text{Reducing power} = [(A_c - A_0) / A_0] \times 100$$

A₀ = absorbance of sample

A_c = absorbance of added sample concentration

DPPH Scavenging Assay

DPPH scavenging activity of both samples was assessed by using sultana method. 25µg methanol was added into Sample, and then freshly prepared DPPH solution was mixed to upper forming solution and kept it for 30 minutes in dark. Then absorbance of samples was determined by spectrophotometer at 517nm. Low absorption of sample indicates high scavenging activity. Percentage inhibition of DPPH scavenging activity was determined by following formula:

$$Q = [(A_c - A_0) / A_0] \times 100$$

A₀ = absorbance of sample

A_c = absorbance of added sample concentration

Preparation of Transdermal Patches

Baking layer: 4g of PVA (polyvinyl alcohol) + 100ml of water were heated at 80 °C for 2 hours at magnetic stirrer. The methanol 15ml in a beaker was taken and 50mg HPMC (hydroxyl propyl methyl cellulose) was added into methanol and stirrer by using magnetic stirrer for 30 minutes. Then 50mg eudragit was added into solution and again stirrer for 30 minutes. Emulsion A and B were added separately and again stirrer for 30 minutes. After this, poly ethylene glycol was added and mixed it for 20 minutes and sonicate it for 20 minutes. Then it was poured into baking layer and placed into an oven for dry it at 35C for 48 hours. After drying, peeled them off with the help of blade from surfaces of Petri dishes, cut into desired size. These transdermal patches were used on wounds of rabbits.

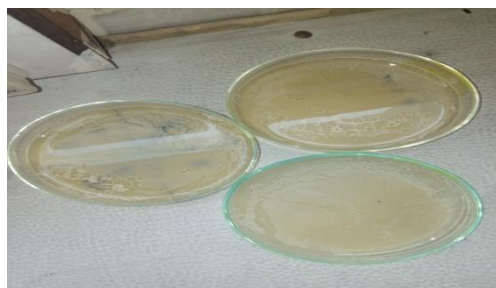


Figure 1: Preparation of transdermal patches.

Development of wound

Twelve rabbits weighting 2-3 kg was taken from University of agriculture Faisalabad. Rabbits were housed in separate cages and were kept for one week. The feed was given twice daily at morning and at evening and water was given throughout the day. Rabbits were dividing into three groups and then anesthetized by administration of lignocaine (local anesthetic) then dorsal skin of rabbit was clipped to remove hairs on skin. After removing hairs, the skin was cleaned with spirit and incisions (4 cm² areas, 0.5 cm² thicknesses) was produced by using sterilized

and sharp surgical blade on skin to check wound healing activity of transdermal patches.



Figure 2: Application of patches on animals.

Statistical analysis

Data was expressed with mean±SE (standard error) and was analyzed by graph pad prism or SPSS to find significant difference among groups. A value of $p < 0.05$ was used for statistical significant.

RESULTS

Transdermal patches were synthesized by using baking layer and matrix loading with drug as shown in figure 1.

In-vitro Antibacterial Activity of Patches

In vitro antibacterial activity of preparation of castor oil + acetone and castor oil + acetone+ antioxidant was determined against two bacterial strains i.e. one gram negative strain and one gram positive strain. The results were in term of MIC as tabulated in table.



Figure 3: MIC test for *E. coli*.

Table 3 shows the result of antibacterial activity of transdermal patches formulation against two bacterial strains. 50µg/ml concentrations of bacteria were used to calculate the minimum inhibitory concentration. Patch A showed 4µg/ml and patch B showed 32 µg/ml MIC against *E. coli* respectively. Patch A showed 2µg/ml and patch B showed 64 µg/ml MIC against *S. aureus* respectively. Ampicilline antibiotic was used as a standard showed 16µg/ml against *E. coli* and 32 µg/ml MIC against *S. aureus*. The results showed that antibacterial activity of patches A

containing castor oil and acetone emulsion shows better activity as compare to patch B containing castor oil, acetone and vitamin C and E emulsion.

TABLE 3: MIC of formulation and standard drug against *E. coli* and *S. aureus*.

Formulation	<i>E. coli</i>	<i>S. aureus</i>
Castor oil + acetone emulsion containing patches	4µg/ml	2 µg/ml
Castor oil + acetone+ antioxidant emulsion containing patches	32 µg/ml	64 µg/ml
Standard drug	16 µg/ml	32 µg/ml

Total Phenolic Contents

Total phenolic content of the different extracts of emulsion were calculated by using the modified Folin-Ciocalteu method and the results are expressed as gallic acid equivalent. The result of total phenolic content of the different extracts of turnip peels are given in Fig 4.

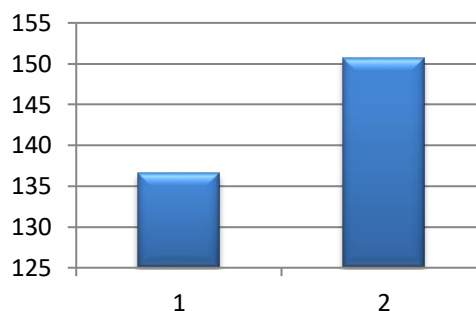


Figure 4: TPC of both patches formulations.

Total Flavonoids Contents (TFC)

Total flavonoid contents of the preparations were measured spectrophotometrically by using aluminium chloride colorimetric method. The results of total flavonoid contents are expressed as catechin equivalent. The results of total flavonoid contents are given in Fig 5.

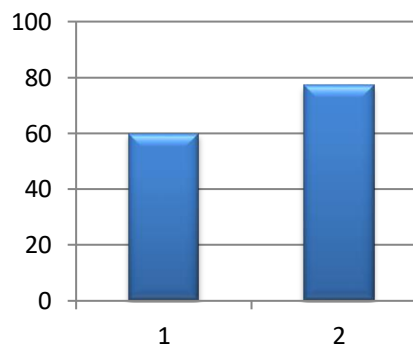


Figure 5: TFC of both patches formulations.

Reducing Power Assay

Results of reducing power assay is given in Fig 6.

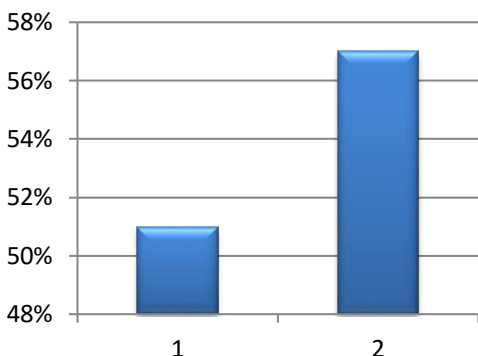


Figure 6: Chart for reducing power assay.

All these results of the ferric reducing power activity are expressed as % reducing activity. While significant difference was observed the reducing power activities. The results of reducing power assay was showed that transdermal patches containig castor oil, acetone and vitamin C and E have more reducing power as compared with patch containing only castor oil and acetone.

DPPH Inhibition Assay

The results of percentage DPPH radical inhibition of formulation A and B are given in Fig. 7.

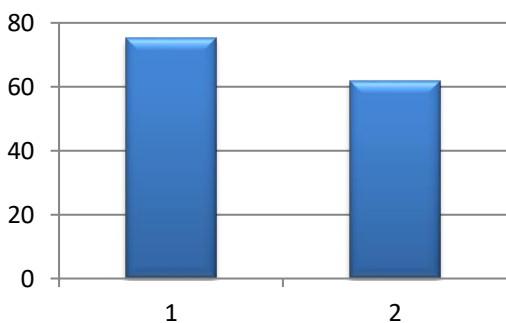


Figure 7: Percent inhibition of DPPH of both formulations.

The table showed the values of percentage DPPH radical inhibition. All the results of DPPH radical scavenging activity are expressed as % inhibition. These results showed that formulation B transdermal patches containig castor oil, acetone and vitamin C and E have more redical scavenging activity as comared with patch containing only castor oil and acetone.

In-vivo Wound Healing Activity

For evaluation of wound healing potential of transdermal patches A and B, wound healing

contraction score was measured and calculated after every 5 days over a period of 20 days after patches application. Table 4 showed the wound contraction score over a period of 20 days. On 1st day of experiment after application of transdermal patches to all groups the wound area for patch A and B was 4 ± 0.0 cm² and no change was occurred as it was the start of treatment. On the 5th day of treatment the wound area was calculated for patch, B and control group that was for control group 3.7 ± 0.0 cm², for transdermal patch A 3.6 ± 0.04 cm² and for transdermal patch B it was 3.4 ± 0.06 cm². On the 10th day of treatment the wound area was calculated for patch, B and control group that was for control group 3.0 ± 0.06 cm², for transdermal patch A 2.7 ± 0.05 cm² and for transdermal patch B it was 2.7 ± 0.07 cm². On the 15th day of treatment the wound area was calculated for patch, B and control group that was for control group 2.4 ± 0.03 cm², for transdermal patch A 2.2 ± 0.04 cm² and for transdermal patch B it was 2.0 ± 0.05 cm². On the 17th day of treatment the wound area was calculated for patch, B and control group that was for control group 2.1 ± 0.05 cm², for transdermal patch A 1.7 ± 0.05 cm² and for transdermal patch B it was 1.6 ± 0.05 cm². On the 20th day of treatment the wound area for control group 0.8 ± 0.07 cm², for transdermal patch A 0.1 ± 0.01 cm² and for transdermal patch B it was 0.0 ± 0.00 cm². These calculations confirm that the healing process is fast in transdermal patches of emulsion B that contain antibacterial and antioxidant properties also. The control group shows very slow process of healing of wound as it was present till last day of observation.

Table 4: Wound contraction score observed within 20 days.

Days	GROUP 1	GROUP 2	GROUP 3
	Control group 4cm ²	Treatment 1 4cm ²	Treatment 2 4cm ²
0	4 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
5	3.7 ± 0.0	3.6 ± 0.04	3.4 ± 0.06
10	3.0 ± 0.06	2.7 ± 0.05	2.7 ± 0.07
15	2.4 ± 0.03	2.2 ± 0.04	2.0 ± 0.05
17	2.1 ± 0.05	1.7 ± 0.05	1.6 ± 0.05
20	0.8 ± 0.07	0.1 ± 0.01	0.0 ± 0.00

Data was analyzed by using two ways ANOVA. Each group was compared with control. $p < 0.05$ ns: non-significant

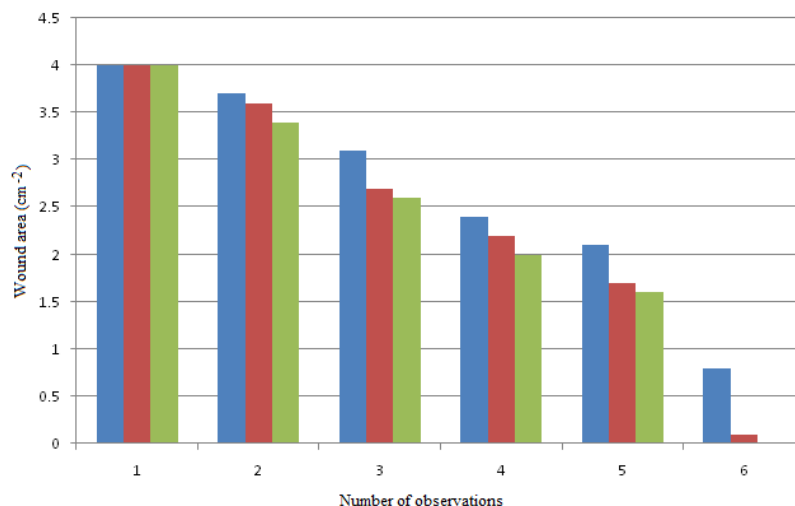


Figure 8: Rate of wound contraction of control patch formulation A and formulation B.
Blue bar indicates control group
Red bar indicates treatment group 1 containing only castor oil and acetone containing emulsion
Green bar indicates treatment group 1 contains only castor oil, acetone and antioxidant containing emulsion

DISCUSSION

Tranfermal Patches

Tranfermal patches were synthesized by using castor oil and acetone emulsion and second preparation was prepared for comparison with castor oil, acetone and vitamin C and E emulsion.

These tranfermal patches were used for further testing of antioxidant, in vitro antibacterial activity and in vivo wound healing properties.

Antioxidant Properties

Oxidation is very important to various living organisms because it generate energy for biological processes. ROS as well as other oxygen centered free radicals are produced constantly in body of living organism the cause tissue damage and death of cell. The oxygen radical's cause illness, comprising cancer and cardiovascular illnesses, ageing, diabetes etc [8]. Natural antioxidants might protect oxidative damage. Antioxidants are present mostly in dietary plants and therapeutic plants [9]. ROS or free radicals have been involved in various diseases processes. Aerobic breathing, lipid per oxidation as well as inflammatory process are involved in the production of free radicals. Antioxidants then protect or reduce harmful actions of these. For severe constant oxidative stress the intrinsic defense mechanism of body may not be sufficient. Hence, exogenous antioxidants are used to delay or inhibit the actions of free radicals in human body to prevent damage. Synthetic antioxidants are mostly used in industrial procedures [10]. Many diseases are associated with oxidative stress caused by free radicals that are formed by the reaction of

oxygen molecules to form charge particles. The present study evaluated the *in vitro* antioxidant and antibacterial activities of various extracts of *R. communis* (castor oil). *In vitro* antioxidant activities of the plant extract were determined by DPPH, total flavonoid contents, total phenolic contents and reducing power assay. Total phenolic contents of these plants were determined by gallic acid equivalent (GAE).Plants showed significant radical scavenging activity.

Total Phenolic Contents

Due to the hydroxyl groups phenolic compounds show scavenging activity. Phenolic components are important because they hinder oxidative spoilage of lipids so in this way expand the quality of life. Total phenolic constituents of sample were evaluated as GAE. 45µg GAE total phenolic in 1mg extract was evaluated. phenolic constituents are natural antioxidant and directly link antioxidant action determined that TPC and antioxidant potential was high in intense color vegetables like onion but in low value in watery vegetables. In this research results showed that formulation A having 136.6 mg of GAE and B have 150 mg of GAE.

Total Flavonoid Contents

Previous studies show that flavonoid contents show very good antioxidant properties. Drmaain claimed that flavonoids exert anti-proliferative action which could modulate lymphocytes activation. He investigated that TPC, TFC, and iron chelating agents are present in some medicinal plants.

Epidemiological investigation on flavonoid containing foods suggested that eating of food rich in flavonoid prevent coronary heart diseases and cancer [11].

This research shows that transdermal patches containing castor oil and acetone show 59.86 TFC, while transdermal patches containing castor oil, acetone and vitamin E and C shows 77.23 TFC value.

DPPH Scavenging Activity

It is very stable organic free radical and it has deep violet color which gives absorption maximum at 515-528 nm. When concentration of phenolic compounds and degree of hydroxylation increases DPPH scavenging activity also increase [12, 13]. Essential oil and ethanolic extracts present excellent DPPH scavenging activity. It shows more activity with absolute alcohol then 80% methanol or ethanol extract. Synthetic components show very slightly DPPH scavenging activity then essential oils [14, 15]. It was observed that sample B transdermal patches containig castor oil, acetone and vitamin C and E have more redical scavenging activity as compared to patch containing only castor oil and acetone.

Reducing Power Assay

This assay used to determined antioxidant activity of different food materials like plant extracts and nutritional supplements. In reducing power assay determination technique, ferric ion reduces to ferrous ions and change from yellow to bluish color. More intense color indicates more reducing power of components. As intensity of color is high there will be high absorption as a result greater will be the antioxidant activity of compound. The results of reducing power assay showed that transdermal patches containig castor oil, acetone and vitamin C and E have more reducing power as comared with patch containing only castor oil and acetone.

***In-vitro* Antibacterial activity**

R. communis has activity against Streptococcus species, klebsiella pneumonia, candida albicans, proteus vulgaris, Staphylococcus aureus and Escherichia coli. Petroleum ether and acetone extracts and petroleum ether have excellent zone of inhibition but ethanolic extract show anti-bacterial activity only at higher concentration (Immunocompromized Patients suffering with oral cancer develop secondary infections due to different bacterial and fungus species. These infections are treated with co-administration of *R. communis* with the immunosuppressant drugs [6].

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patches were prepared and their antiabacterial activity was checked by using minimum inhibitory concentration dilution method. It was found that patch A showed good antibacterial activity then patch B and results also shows that patch A containing castor oil and acetone emulsion shows excellent antibacterial activity against *S.aureus* then *E.coli*. All bacterial strains were inhibited by sample A that shows that castor oil with acetone emulsion shows better antibacterial activity then sample B.

***In-vivo* Wound Healing Activity of Trandermal Preparation**

The castor oil of *R. communis* seed has antiulcer activity at a dose of 500 mg/kg and 1000 mg/kg, but according to different experiments the dose 1000 mg/kg was more effective against the ulceration caused by aspirin, *H.pylori*, and ethanol in rats. The antiulcer activity of *R. communis* is due to the cytoprotective action of the drug or strengthening of gastric mucosa and thus enhancing the mucosal defence against *H.pylori* and ulcer causing NSAID like aspirin [5]. Results showed the wound contraction score over a period of 20 days. The calculations confirm that the healing process is fast in transdermal patches of emulsion B that contain antibacterial and antioxidant properties also. The control group shows very slow process of healing of wound as it was present till last day of observation.(table no 9 and 10)

CONCLUSION

It is concluded from this research the emulsion of castor oil and acetone (emulsion A) and emulsion of castor oil, acetone and vitamin E and C (emulsion B) was prepared by titration method with help of pestle and motor. Antioxidant properties were check of these patches that shows good results with emulsion B. *In-vitro* antibacterial activity of these patches were check against *E. coli* and *S. aureus* bacteria that showed transdermal patches having emulsion A posses good results. Transdermal patches were loaded with these two emulsions to check in vivo wound healing properties that prove that patches having emulsion B shows good wound healing properties due to having both activities of antibacterial and antioxidant properties. All these properties make these patches a suitable candidate for wound healing dressing's materials. These patches have antibacterial and antioxidant properties also so wound healing process will be fast.

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