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The efficacy of aqueous extract of 2 herbs viz, ROSA DAMASCE NA and OCIMUM SANCTUM

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Abstract:
Introduction: *Recurrent aphthous ulcers or stomatitis or RAU / RAS is the most common oral mucosal diseases [(1] , whose aetiology remains largely unknown [(2)]. Plants are one of the most important sources of drugs and their medicinal use has a long history. The plant medicines have traditionally been known to have soothing, analgesic and antibacterial properties. and lesser side effect to substitute the current chemical therapy.*
Aim: *To compare the efficacy of Aqueous extracts of Rosa damascena and Ocimum sanctum in healing and pain relief of aphthous ulcers*
Materials and Methods: Aqueous extracts of Rosa damascena and Ocimum sanctum, placebo
Results: *Ocimum sanctum, showed better results in terms of reduction in size of ulcers and pain, as compared to Rose extract and placebo*
Clinical significance: Use of these extracts in the form of oral rinses provides simple highly beneficial, and cost effective therapy, due to absence of side effects, these preparations can be used for recurrent episodes of RAS.
Keywords: *oral ulcers, rose, tulsi, Rosa damascena, Ocimum sanctum, pain, plant remedies*

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I. Introduction

Recurrent aphthous ulcers or stomatitis or RAU / RAS is the most common oral mucosal diseases [(1] , whose aetiology remains largely unknown [(2)]. It can affect both men and women of all ages, races and geographic regions [(1)]. It is one of the most painful oral mucosal inflammatory ulcerative conditions and can cause pain on eating, swallowing and speaking [(3)]. All systemic and topical therapy is directed at relief of symptoms and acceleration of healing. Most systemic medications have side effects which limit their general use [(1)].

Plants are one of the most important sources of drugs and their medicinal use has a long history. Literature reviews indicate that therapeutic use of plants goes back to 4000-5000 B.C. [(4)]. The plant medicines have traditionally been known to have soothing, analgesic and antibacterial properties. and lesser side effect to substitute the current chemical therapy.

Rosa damascena or Persian rose is a small plant belonging to Rosaceae family, with aromatic light pink flowers [(5)]. This plant contains carboxylic acid, terpenes, myrcene, vitamin C, flavonoids like kaempferol, and quercetin glycosides and terpenoids like geraniol, linalool, eugenol, citranellol and farnesol [(6,7,8)] all of which contribute to its high antioxidant property [(6,7,8)].

Tulsi (Ocimum sanctum) has been described, as sacred and as a medicinal plant in ancient literature [(9)]. The name Tulsi is derived from Sanskrit, which means *"The matchless one"* [(10)]. Tulsi has been used as a medicinal herb for thousand years without any known adverse effects.

Our objectives were to evaluate whether traditional plant remedies can be used in routine dental practice for treatment of recurrent oral ulcers; to evaluate the effectiveness of the extracts of Rosa damascena for the symptomatic relief of recurrent aphthous ulcers; to evaluate the effectiveness of the extracts of Ocimum sanctum for the symptomatic relief of recurrent aphthous ulcers; to evaluate the effectiveness of the extracts of Rosa damascena versus Ocimum sanctum for the symptomatic relief of recurrent aphthous ulcers.

This was a randomized, placebo-controlled clinical trial with 60 patients being selected from those attending the OPD of YMT Dental College and Hospital, Navi Mumbai. Each patient was given a detailed description of the study and the procedure and was required to sign an informed consent form before participation. The study was in accordance with the Helsinki Declaration of 1975, as revised in 2000. The ethical committee from MUHS, Maharashtra, approved the study protocol, patient information sheet, and informed consent.

The following patients were included in the study

1. The patient should present with at least one aphthous ulcer measuring no more than 10 mm in diameter, and the duration of the ulcer(s) should have been no more than 48 to 72 hours.
2. Patients with minor or herpetiform ulcers
3. Patients ranging from 18 to 60 years of age with a history of recurrent aphthous ulcers.

Patients with a history of systemic diseases or immunologic diseases like Behcet's disease, Reiter's syndrome, those with long standing infections, patients with history of use of non-steroidal anti-inflammatory drugs [NSAIDS], corticosteroids or other immunomodulatory agents, within one month prior to the study, patients younger than 18 years of age were excluded from the study. For the purpose of our study, the following oral rinses were used.

1. Oral rinse containing aqueous extract of Rosa damascena and water in the concentration of 1:4 [20%].
2. Oral rinse containing aqueous extract of Ocimum sanctum and water in the concentration of 1:4 [20%].
3. Oral rinse of distilled water as placebo

The experimental oral rinses were obtained from the Sugandhim Co. Pvt. Ltd., Mumbai. They were colourless liquids, with characteristic fragrances. However, we did not try to mimic the fragrance of the experimental mouth rinses in placebo oral rinse. Trying to mimic or simulate the odour would have led to the addition of other chemical constituents which could have confounded the results.

Three groups of 20 patients each were made, namely Group A, Group B, and Group C , by using a computer-generated random numbers list.

1. Patients in Group A received oral rinse containing aqueous extract of Rosa damascena and water in the concentration of 1:4 [20%].
2. Patients in Group B received oral rinse containing aqueous extract of Ocimum sanctum water in the concentration of 1:4 [20%].
3. Patients in Group C received oral rinse of distilled water as placebo

Examination of the ulcers was done on baseline i.e. day 0, using a calibrated periodontal probe, with millimetre markings, using adequate illumination. The size (measured as the surface area) and the number of ulcers was noted. Pain associated with the ulcers was determined using the visual analogue scale (VAS) ranging from 1 to 10.

The size of the ulcers in case of round ulcers was measured as the largest diameter. The surface area of these ulcers was calculated using the formula 3.14 x (Radius) ^{2}. The size of the ulcers in case of ovoid ulcers, was measured as **a** (largest diameter) x **b** (smallest diameter perpendicular to the largest diameter). The surface area of these ulcers was calculated using the formula 3.14 x a x b.

In the case where there was more than one ulcer in the oral cavity, the surface area was calculated for each ulcer, according to the formula given above and the sum of the individual surface areas was taken as the surface area for that patient.

The follow up was done on day 4 and then on day 7. The surface area was denoted as S0 on day 0, S4 on day 4 and S7 on day 7 to denote the change in size from which the 'Efficacy Index' was calculated per patient.

Patients belonging to each group were instructed to rinse with 5ml of the given mouthwash, for 30 seconds, with thorough swishing, four times in a day, preferably after oral hygiene procedures to enhance the efficacy of the rinse. Healing was measured using following parameters.

1. Ulcer size
2. Number of ulcers
3. Pain experienced by the patient

Meng [(2)] et al, described a formula based on the size of the ulcer, to calculate the efficacy index of the treatment.

$$\text{EFFICACY INDEX in \%} = \frac{(S0 - S4/\ S7)}{S0} \times 100$$

Efficacy index was calculated for all 60 patients individually.

Patients were regularly contacted and encouraged to comply with the treatment protocol. At each visit, subjects were asked about any adverse effects or complications such as taste alteration or hypersensitivity, abnormal changes in the oral mucosa and to report any such occurrences to the investigators immediately.

After completion of data accumulation, the results were tabulated and statistical analysis was done using the independent sample test and Mann-Whitney test.

22 males and 38 female patients were selected from the OPD out-patient department of Oral Medicine and Radiology. Only those patients with a positive history of recurrent aphthous ulceration were selected for the study.

The ulcers were graded according to:

1. The Number of ulcers
2. The size of the ulcer- calculated as the surface area and its Efficacy Index./ the Efficacy Index of its surface area.
3. The Visual Analog Scale (VAS) score

II. Results:

In our study, there was a female predominance in the population being studied. Our study comprised of 38 (63.33 %) females 22 (36.67 %) males.

GROUP A consisted of 5 males (25%) and 15 females (75%) and the mean age was 25 years. Day 0 mean number of ulcers was 1.40, and the mean VAS score was 5.05. Day 4 mean number of ulcers was 1.35, mean efficacy index was 42.31, and mean VAS score was 3.20. Day 7 mean number of ulcers was 0.55, mean efficacy index was 85.72, and mean VAS score was 1.10.

GROUP B consisted of 9 males (45%) and 11 females (55%) and the mean age was 27.15 years. On Day 0, the mean number of ulcers was 1.60, and the mean VAS score was 4.53. Day 4 mean number of ulcers was 1.55, mean efficacy index was 45.66, and mean VAS score was 2.74. Day 7 mean number of ulcers was 0.55, mean efficacy index was 86.54, and mean VAS score was 0.84.

GROUP C consisted of 8 males (40%) and 12 females (60%) and the mean age was 28.75 years. On Day 0, mean number of ulcers was 1.75, and the mean VAS score was 4.80. Day 4, mean number of ulcers was 1.55, mean efficacy index was 32.86, mean VAS score was 2.95. Day 7, mean number of ulcers was 0.90, mean efficacy index was 61.68, mean VAS score was 1.50.

III. Discussion

Recurrent aphthous stomatitis, one of the most common painful oral mucosal conditions seen presents as recurrent, multiple, round or ovoid ulcers with circumscribed margins having a yellow or gray floor and surrounded by an erythematous halo [(14)]. These ulcers have been postulated as being multifactorial in origin such as emotional stress, infections, hormonal changes, and immunodeficiency and vitamin deficiencies, posing a challenge to the oral physician.

NUMBER OF APHTHOUS ULCERS

Comparison of the number of ulcers between group A and group B and group C was not statistically significant at the end of Day 7 (p value > 0.05). This finding was in contrast with the study conducted by Hoseinpour et al, 2011 [(5)] our study used an aqueous extract of Rosa damascena along with water. The authors used a mouthwash containing aqueous extract of Rosa damascena along with glycerine 2% by weight, sodium polycarboxymethylcellulose 0.5%, polysorbate 80 0.025% by weight, saccharin sodium 0.05% by weight, distilled water 20 ml, and R. Damascena extract 20% by weight. Their result may be attributed to the presence of other constituents which may have enhanced the properties of Rosa damascena.

Comparison between group A and group B did not show a statistically significant difference. comparison between group A and group C, did not show a statistically significant difference as $p = 0.192$ on day 4; but showed that there was a statistically significant difference as $p = 0.022$ on day 7. This finding correlates with the study conducted by Hoseinpour et al 2011 (6).

Comparison between group B and group C, did not show a statistically significant difference as $p = 0.076$ on day 4; but showed that there was a statistically significant difference as $p = 0.018$ on day 7. However, the response to healing received for experimental group B was marginally better than that received for group A, which may be attributed to eugenol content, caryophyllene, cyclohexane exerting a more pronounced antimicrobial and anti-inflammatory action of Ocimum sanctum.

Essential oils of *Rosa damascena*, which contain polyphenolics and flavonoids [(6, 7, 8, 17, 18)], were tested in vitro, by **Lisin G, et al, 1999** [(19)], for antimicrobial activity against gram-positive *Staph. aureus*), gram negative *E. coli* , and yeast *Candida albicans*); the essential oils exhibited inhibitory and bactericidal activities against all tested microorganisms at low concentrations and also antioxidant activity. Antibacterial effect of some of the major components of rose oil (citrenellol, geraniol and nerol) was reported recently by **Andoğan BC et al , 2008; Gochev V et al, 2008** [(20,21)].

O.sanctum has essential oils which are capable of killing Gram-positive and –negative microorganisms as noted earlier. O. Sanctum [(11-13,22-24)] is a rich source of polyphenolic, flavonoids, and phenolic compounds and has antioxidant and free-radical scavenging activity, which by virtue of scavenging the reactive oxygen species may have contributed to faster healing of the aphthous ulcers.

REDUCTION IN VAS SCORES

The VAS score in the experimental group B (O. sanctum) was showed better improvement in comparison to experimental group A (R. damascena) and control group C (placebo). p =0.673 between groups B and C on day 4 and p= 0.659 (i.e.>0.05) between groups A and C on day 4; p= 0.180 between groups B and C on day 7 and p=0.442 between groups A and C on day 7.

Antioxidant and free radical scavenging activity has been shown in various extracts of Rosa damascena [6, 7, 8, 17, 18] and Ocimum sanctum [11-13, 23, 24] due to their rich source of polyphenolic, flavonoid and phenolic compounds. Several authors have proposed that a possible relationship exists between the inflammatory process and the free radical metabolism. Molecular oxygen plays a central role in the pathogenesis and therapy of chronic wounds. Over production of reactive oxygen species (ROS), causes oxidative stress, leading cytotoxicity and delayed wound healing [25]. Any provided treatment must aim to reduce this oxidative stress.

Rosa damascena is a rich source of polyphenolic flavonoids. R. damascena contains vitamin C, folate per 100 g in rose hips contained more than fresh rose hip (Stralsjö et al., 2003), which has antioxidant and anti-inflammatory effects as per Maleev A, et al, 1972 and; Tannenbaum SR, et al, 1991. Kumar et al, 2009, have proved that Rosa damascena had higher antioxidant property when compared with L-ascorbic acid [26-28].

Antibacterial property exhibited by both plants could have contributed to the faster, better healing time. Phenolic compounds in Rosa damascena and Eugenols in Ocimum sanctum could have contributed to reduction in secondary bacterial infection of the oral ulcers and contributed to faster alleviation of pain and faster rate of healing. Ng, Ulusoy et al in 2009 [17] showed antibacterial activity of essential oil against Escherichia coli, Pseudomonas aeruginosa, B. subtilis, Staph. aureus, Chromobacterium violaceum. The other constituents of rose i.e. Phenylethyl alcohol, Citrenellol, Geraniol and Nerol contained in the Hydrosol possessed anti-microbial properties was reported recently by **Andoğan BC et al , 2008; Gochev V et al, 2008** [20-21]. Eugenol has strong antibacterial and antifungal properties.

Analgesic property may be due to the high tannin content of the R, damascena and O.sanctum extracts. In RAS, pain is due to secondary bacterial infection and trauma from the oral cavity. Tannins are polyphenolic compounds of high molecular weight which form complexes with salivary proteins to form a physical barrier film over the ulcers aiding in pain reduction and healing by decreasing exposure to irritants[5].

Eugenol acting along with other phytochemicals, contained in O. sanctum, acts as a local antiseptic,anaesthetic, ananlgesic effect, along with caryophyllene which is anti-inflammatory.

Adaptogenic property [22] has been found exclusively with Ocimum sanctum by which the plant substance increases the state of non-specific resistance against multiple stresses. Animal studies using [ethanolic] extracts of Ocimum sanctum have shown increased physical endurance and better healing from experimentally induced ulcers [23,29] (using aspirin tablets in a dose of 20mg/kg.), experimentally induced excision, incision, dead space wounds models and dexamethasone-suppressed wound healing model in animals [30]. It has been shown that there is an increase in production of adrenaline, noradrenaline and a decrease in dopamine and serotonin levels resulting in a decrease in inflammation[22].

In animal experiments conducted by **Dharmani P, et al, 2004**, **Goel RK** et al, 2005, ; **R. K. Kath and R. K. Gupta - 2006** [23, 31, 29], on gastric ulcers using Ocimum sanctum, they found that Ocimum sanctum extract, decreased the incidence of ulcers and also enhanced the healing of ulcers. Ocimum sanctum extract in a dose of 100 mg/kg was found to be effective and significantly reduced free, total acidity and peptic activity, and increased mucin secretion by 34.61%. They deduced that the secretion of mucin might play a small but contributory role in healing of the gastric ulcers; hence it can be postulated that a similar mechanism i.e. up regulation of tissue defence factors may help in the overall healing of oral aphthous ulcers. The tannins form complexes with salivary proteins and create a protective barrier over the ulcers thereby speeding the healing and preventing the exposure to irritants in the oral cavity and helps minimize the pain. In a study conducted by Hoseinpour et al 2011 [5], there was a rapid relief of pain in patients receiving Rosa damascena extract which they attributed to the presence of tannins. On the other hand, O. Sanctum has a high content of Eugenol, Cyclohexane, and Tannins. Eugenol contributes to pain relief and its potent anti bacterial properties helps in faster recovery of recurrent aphthous ulcers. Cyclohexane along with tannins helps form a protective barrier, thereby preventing the exposure of the ulcer to irritants. Hence O. Sanctum scores over R. Damascena in rapid relief of pain, reduction of secondary bacterial infection and healing process.

IV. Summary And Conclusion

Ocimum sanctum, showed better results in terms of reduction in size of ulcers and pain associated when compared with the other experimental group and control group. Traditional medicine is widely used and accounts for 40% of all health care delivered. Herbal medicines have good values in treatment. Use of these extracts in the form of oral rinses provides simple highly beneficial, and cost effective therapy, due to absence

of side effects, these preparations can be used for recurrent episodes of RAS. In a developing country like India, such medicinal plants like Rosa damascena and Ocimum sanctum are easily available and cheap to use, indicating need for further research and development to provide the medicinal value of these to the masses.

1. Rosa Damascena
2. The Plant Of *Rosa Damascena*
3. Ocimum Sanctum
4. The Plant Of *Ocimum Sanctum*
5. Aqueous Extract Of Rosa Damascena With Dispensing Vial
6. Aqueous Extract Of Ocimum Sanctum With Dispensing Vial
7. Distilled Water Or Placebo With Dispensing Vial
8. Measurement Of Ulcer Size Using A Calibrated Periodontal Probe
9. Ulcers Treated With Aqueous Extract Of Rosa Damascena
10. Ulcers Treated With Aqueous Extract Of Ocimum Sanctum
11. Ulcers Treated With Distilled Water Or Placebo

1. ROSA DAMASCENA
2. The plant of *Rosa damascena*
3. OCIMUM SANCTUM
4. The plant of *Ocimum sanctum*
5. AQUEOUS EXTRACT OF ROSA DAMASCENA WITH DISPENSING VIAL
6. AQUEOUS EXTRACT OF OCIMUM SANCTUM WITH DISPENSING VIAL
7. DISTILLED WATER OR PLACEBO WITH DISPENSING VIAL
8. MEASUREMENT OF ULCER SIZE USING A CALIBRATED PERIODONTAL PROBE
9. ULCERS TREATED WITH AQUEOUS EXTRACT OF ROSA DAMASCENA
10. ULCERS TREATED WITH AQUEOUS EXTRACT OF OCIMUM SANCTUM
11. ULCERS TREATED WITH DISTILLED WATER OR PLACEBO

Which one is preferable madam? 1st or the 2nd

12. ROSA DAMASCENA
13. The plant of *Rosa damascena*
14. OCIMUM SANCTUM
15. The plant of *Ocimum sanctum*
16. AQUEOUS EXTRACT OF ROSA DAMASCENA WITH DISPENSING VIAL
17. AQUEOUS EXTRACT OF OCIMUM SANCTUM WITH DISPENSING VIAL
18. DISTILLED WATER OR PLACEBO WITH DISPENSING VIAL
19. MEASUREMENT OF ULCER SIZE USING A CALIBRATED PERIODONTAL PROBE
20. ULCERS TREATED WITH AQUEOUS EXTRACT OF ROSA DAMASCENA
21. ULCERS TREATED WITH AQUEOUS EXTRACT OF OCIMUM SANCTUM
22. ULCERS TREATED WITH DISTILLED WATER OR PLACEBO

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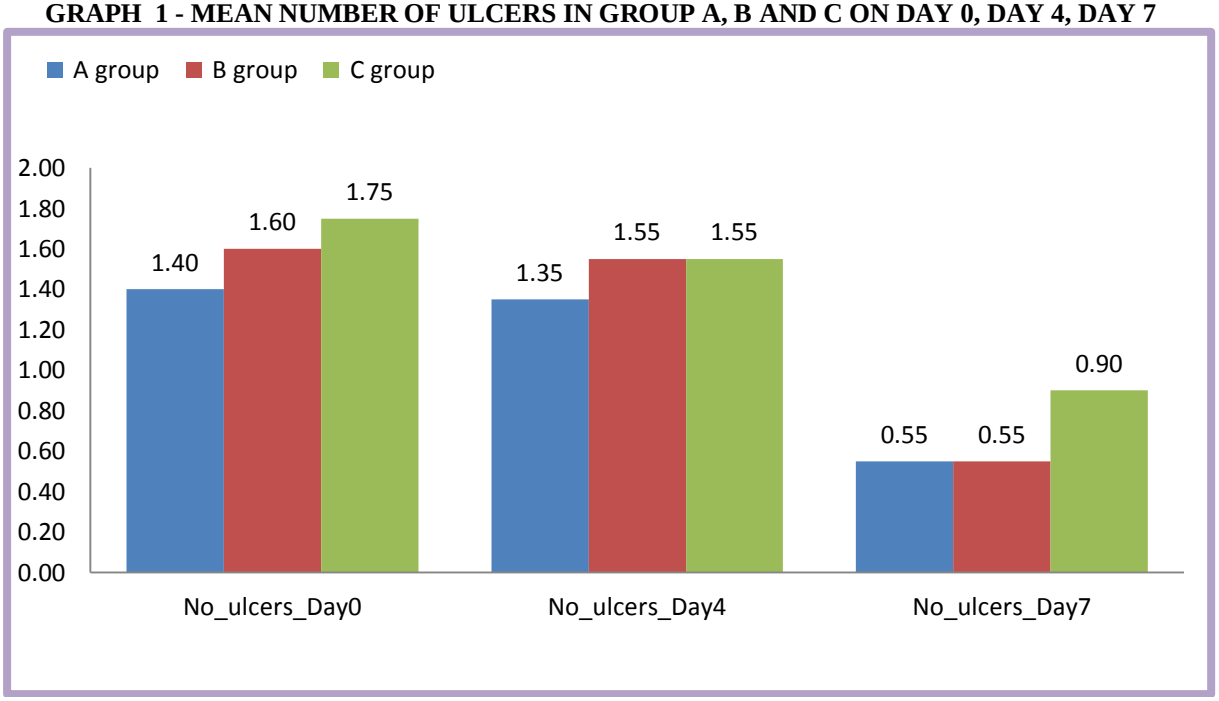
TABLE 1 - COMPARISON OF MEAN NUMBER OF ULCERS USING THREE TREATMENT MODALITIES ON DAY 4 AND DAY 7

GROUP	MEAN NUMBER OF ULCERS DAY 0	MEAN NUMBER OF ULCERS DAY 4	Group	p value Independent Sample test	p value Mann Whitney U test	MEAN NUMBER OF ULCERS DAY 7	p value Independent Sample test	p value Mann Whitney U test
Group A	1.40	1.35	Group A v/s Group B	0.549	0.684	0.55	1.0	0.891
Group B	1.60	1.55	Group A v/s Group C	0.426	0.221	0.55	0.123	0.153
Group C	1.75	1.55	Group B v/s Group C	1.0	0.386	0.90	0.142	0.147

GRAPH 1 - MEAN NUMBER OF ULCERS IN GROUP A, B AND C ON DAY 0, DAY 4, DAY 7



ULCER SIZE

TABLE 2 - COMPARISON OF MEAN NUMBER OF ULCERS USING THREE TREATMENT MODALITIES ON DAY 4 AND DAY 7

GROUP	MEAN NUMBER OF ULCERS DAY 4	Group	p value Independent Sample test	p value Mann Whitney U test	MEAN NUMBER OF ULCERS DAY 7	p value Independent Sample test	p value Mann Whitney U test
Group A	1.35	Group A v/s Group B	0.549	0.684	0.55	1.0	0.891
Group B	1.55	Group A v/s Group C	0.426	0.221	0.55	0.123	0.153
Group C	1.55	Group B v/s Group C	1.0	0.386	0.90	0.142	0.147

GRAPH 2 - MEAN NUMBER OF ULCERS IN GROUP A, B AND C ON DAY 0, DAY 4, DAY 7

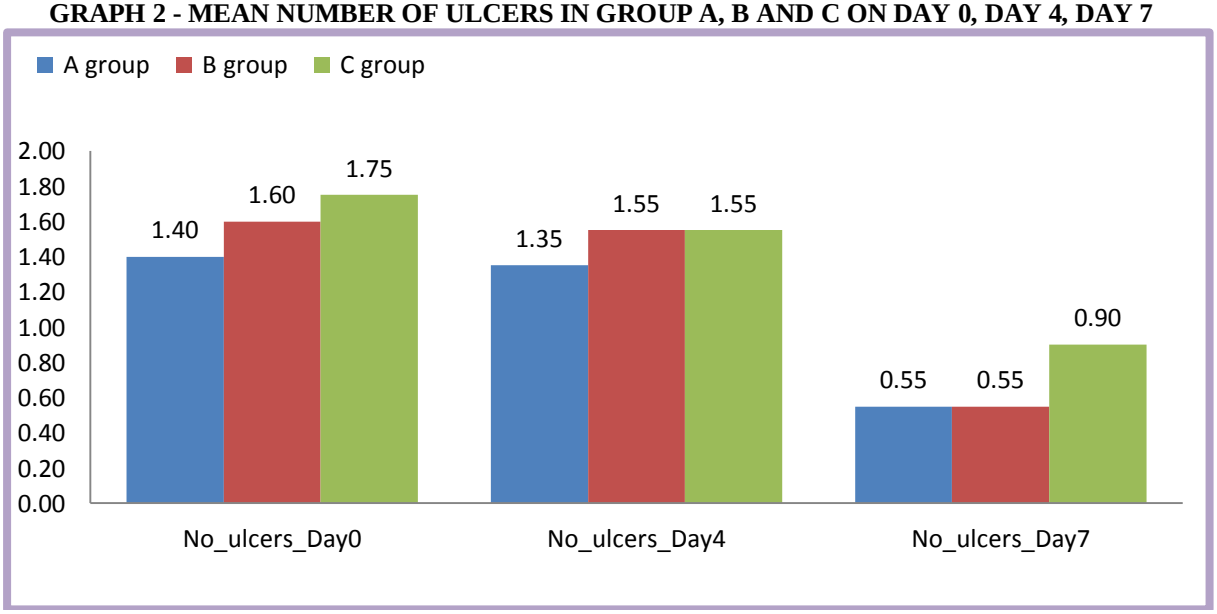


TABLE 3 - COMPARISON OF MEAN EFFICACY INDEX USING THREE TREATMENT MODALITIES ON DAY 4 AND DAY 7

GROUP	MEAN EFFICACY INDEX OF ULCERS DAY 4	Group	p value Independent Sample test	p value Mann Whitney U test	MEAN EFFICACY INDEX OF ULCERS DAY 7	p value Independent Sample test	p value Mann Whitney U test
Group A	42.31	Group A v/s Group B	0.532	0.490	85.72	0.884	0.838
Group B	45.66	Group A v/s Group C	0.192	0.350	86.54	0.022	0.081
Group C	32.86	Group B v/s Group C	0.076	0.207	61.68	0.018	0.063

GRAPH 3 - MEAN EFFICACY INDEX OF CHANGE IN ULCER SIZE IN GROUPS A, B, AND C

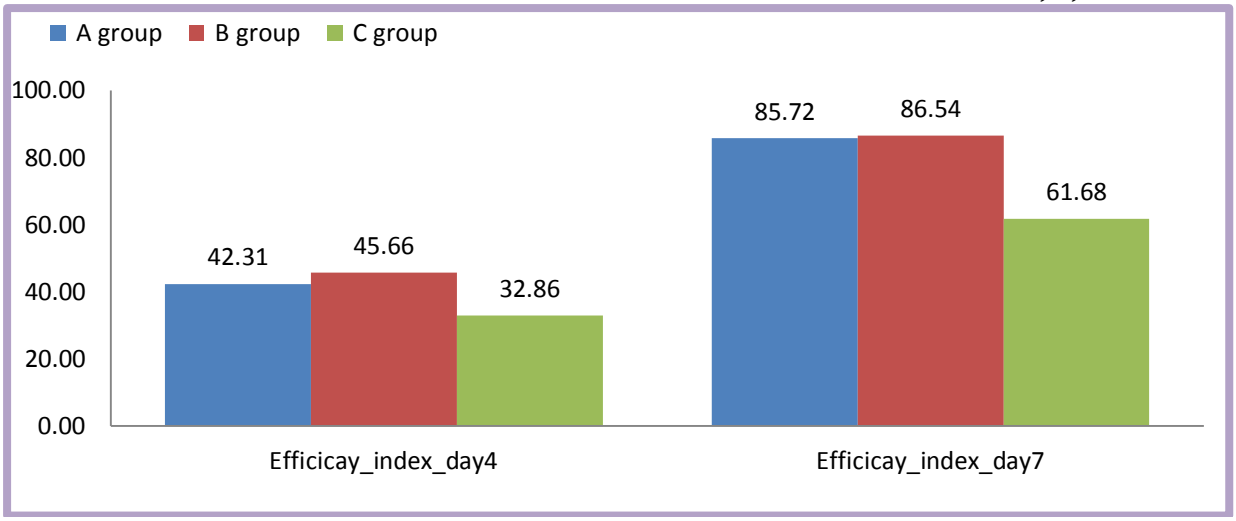
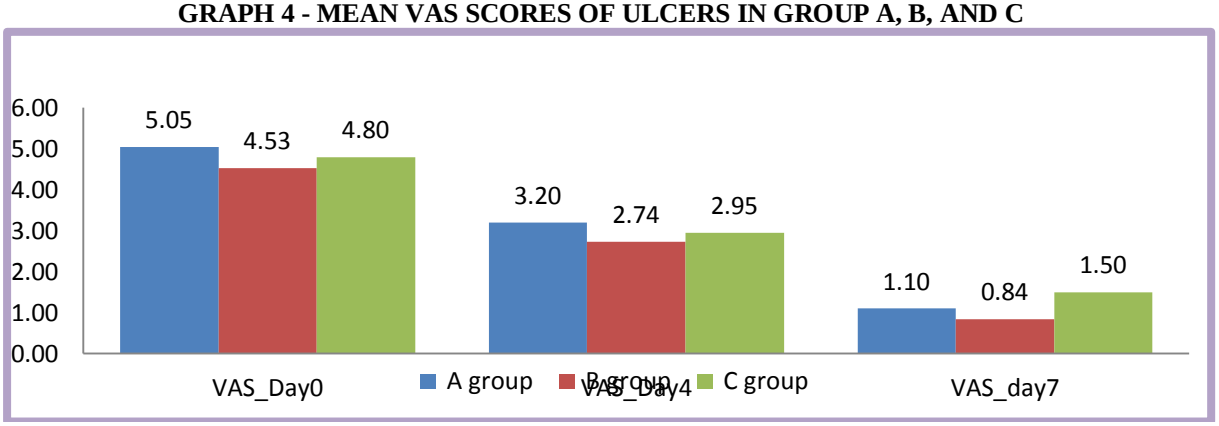


TABLE 4 - COMPARISON OF MEAN VAS SCORES OF ULCERS USING THREE TREATMENT MODALITIES ON DAY 4 AND DAY 7

GROUP	MEAN VAS SCORES OF ULCERS DAY 4	Group	p value Independent Sample test	p value Mann Whitney U test	MEAN VAS SCORES OF ULCERS DAY 7	p value Independent Sample test	p value Mann Whitney U test
Group A	3.20	Group A v/s Group B	0.413	0.584	1.10	0.593	0.708
Group B	2.74	Group A v/s Group C	0.659	0.804	0.84	0.442	0.278
Group C	2.95	Group B v/s Group C	0.673	0.667	1.50	0.180	0.128

GRAPH 4 - MEAN VAS SCORES OF ULCERS IN GROUP A, B, AND C



ROSA DAMASCENA

The plant of *Rosa damascena*

OCIMUM SANCTUM

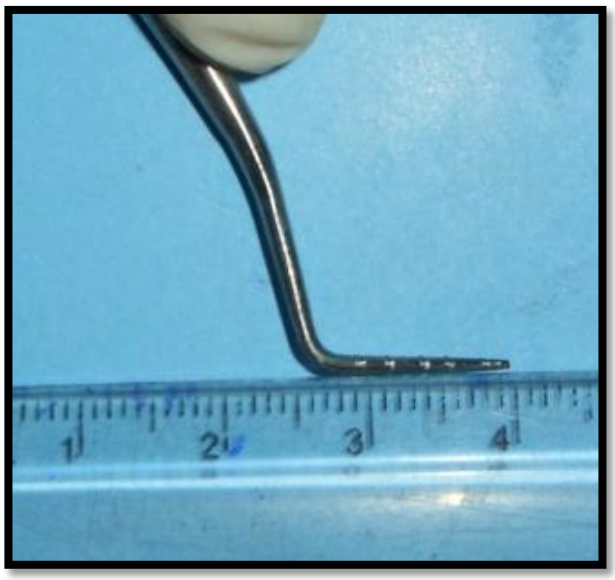
The plant of *Ocimum sanctum*

AQUEOUS EXTRACT OF ROSA DAMASCENA WITH DISPENSING VIAL

AQUEOUS EXTRACT OF OCIMUM SANCTUM WITH DISPENSING VIAL

DISTILLED WATER OR PLACEBO WITH DISPENSING VIAL

MEASUREMENT OF ULCER SIZE USING A CALIBRATED PERIODONTAL PROBE



ULCERS TREATED WITH AQUEOUS EXTRACT OF ROSA DAMASCENA

ON DAY 0

ON DAY 4

ON DAY 7

ULCERS TREATED WITH AQUEOUS EXTRACT OF OCIMUM SANCTUM

ON DAY 0

ON DAY 4

ON DAY 7

ULCERS TREATED WITH DISTILLED WATER OR PLACEBO

ON DAY 0

ON DAY 4

ON DAY 7

Dr. Bhagyashri H. Purandare, et. al. "The efficacy of aqueous extract of 2 herbs viz, ROSA DAMASCENA and OCIMUM SANCTUM." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 16(3), (2021): pp. 62-75.