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EVALUTION OF DIFFERENT EXTRACTS OF ANTI OBESITY POTENTIAL OF CALENDULA OFFICINALIS LEAVES AND BIOCHEMICAL ESTIMATION IN RATS

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ABSTRACT

Obesity is a major global health concern associated with metabolic syndrome, oxidative stress, and hepatic steatosis. The present study evaluated the anti-obesity potential of *Calendula officinalis* (marigold) leaf extracts prepared using different solvents (aqueous, hydroalcoholic, and ethyl acetate) in rats with diet-induced obesity. The extracts were characterized phytochemically for total phenolics, flavonoids, and HPLC profiles. Male Wistar rats were divided into groups: normal diet control, high-fat diet (HFD) control, HFD with low- and high-dose extracts, and a reference drug group (Orlistat). Treatments continued for 8–12 weeks, followed by assessments of body weight, lipid profile, oxidative stress markers, and liver histopathology. Among the extracts, the hydroalcoholic extract showed the most significant reduction in body weight gain, total cholesterol, triglycerides, and LDL levels, while increasing HDL concentrations. It also markedly improved antioxidant status (decreased MDA, increased SOD, GPx, and catalase activity) and ameliorated hepatic histopathological

changes. The anti-obesity effect correlated with the extract's phenolic and flavonoid content. Overall, *C. officinalis* leaf extracts—particularly the hydroalcoholic fraction—exhibited strong anti-obesity and antioxidant activity, likely through modulation of lipid metabolism and oxidative stress. These findings support further mechanistic and clinical evaluation of *C. officinalis* as a potential complementary therapy for obesity.

KEYWORDS: Calendula officinalis, Obesity**,** lipid profile**,** oxidative stress**,** liver histology rat models.

1. INTRODUCTION[1-5]

- Obesity and its comorbidities: epidemiology, metabolic impact (dyslipidemia, NAFLD, oxidative stress, inflammation).
- Limitations of existing therapies.
- Potential role of phytochemicals and medicinal plants in obesity treatment; antioxidants/flavonoids can inhibit oxidative damage, enhance lipid metabolism, etc.
- Calendula officinalis: botanical characterization; folk uses (wound healing, anti-inflammatory, hepatoprotective). Brief summary of existing studies of relevance to lipid metabolism/dyslipidemia

Impact of Calendula officinalis extract on liver histopathology, lipid profile, and oxidative stress in rats placed on a cholesterol and carbohydrate-rich diet revealed modulatory activities on lipid profile and oxidative stress in dyslipidemic rats.

Other research on hepatoprotective or antioxidant activity (e.g., against aflatoxin); phenolic composition; safety/toxicity information.

- Gaps: There are not many studies comparing various leaf extracts; most are flower extracts or hydroalcoholic extract; few studies on pure obesity models; biochemical and histological confirmation required.

- ***Calendula officinalis: A Comprehensive Review of its Medicinal Properties***

Calendula officinalis (Calendula), a member of the Asteraceae family, is often known as English Marigold or Pot Marigold. Marigold is a fragrant herb that has been used in traditional medicine for centuries. Calendula species have been highlighted in order to better understand their diverse biological activities and modes of action. Carotenoids, flavonoids, glycosides, steroids and sterols, quinines, volatile oil, and amino acids are all found in

abundance in this plant. Calendula oil is still used as an anti-tumour agent in medicine, which is also a wound healing agent. Among herbal medicines, Calendula suspension or tincture is used to treat acne locally, reduce inflammation, control bleeding and soothe irritated tissues.

Phytochemistry and Ethnopharmacology

For millennia, people have utilized Calendula officinalis Linn. (CO), a well-known medicinal herb belonging to the Asteraceae family of plants. Flavonoids, triterpenoids, glycosides, saponins, volatile oil, carotenoids, amino acids, steroids, sterols, and quinines are all present in this plant.

Numerous biological effects, including hepatoprotective, wound healing, anti-inflammatory, anti-cancer, anthelminthic, and antioxidant properties, are conferred by these chemical compounds.

It's also used for gastrointestinal, gynecological, ophthalmic, and skin disorders, as well as some burn situations. We have included contemporary studies on the therapeutic uses of Calendula officinalis

Traditional uses of *C. officinalis*

In Europe, the leaves are regarded as diaphoretic andexpectorant, whereas the blossoms are employed as a stimulant, antispasmodic, and emmenagogues.

In England, the decoction of the petals was used as a posset drink for the cure of measles and Smallpox and the fresh juice as a cure for jaundice, constipation, and menstrual flow reduction.

In India, thefloretsare utilized in ointments to healwounds, herpes, ulcers, frostbite, skin damage, scars, and purify the blood. Topically, the infusion of the leaves is used to cure varicose veins.

Medicinal plants with anti-obesity activity

- Over the years, several drugs were used to treat obesity, but most of them have now been taken off due to dangerous side effects48. Orlistat is the only FDA-approved long-term obesity treatment. Steatorrhea is a digestive side effect of this medication.

- Sibutramine, another anti-obesity medicine, was discontinued globally due to increased significant, non-fatal cardiovascular events. Pharmacotherapy failures highlight the need for further obesity treatments49,50.
- Natural products are widely used in healthcare and as dietary supplements. Dietary phytochemicals have recently sparked significant interest as possible therapeutic agents for health promotion and alleviation of obesity and related diseases.
- Plant products have long been a fruitful source for the discovery of new medications, and these are used in the most prevalent naturopathy systems due to their chemical richness and aptitude to work on numerous biological targets. A diverse range of medicinal plants and their active constituents can produce beneficial anti-obesity effects such as Curcuminoids (Curcumin), Lignans (Podophyllotoxin), flavones
- (Apigenin, Luteolin), phenolic acids (o-Coumaric acid, chlorogenic acid), flavanols
- (Quercetin), phytosterols (Diosgenin, Brassicasterol, β-sitosterol), alkaloids (Caffeine), Resins (Capsaicin), Pigments (Malvidin, Pelargonidin)

2. Materials and Methods[6-15]

2.1. Plant material and preparation of extracts

- Collection, identification, voucher specimen.
- Drying and powdering leaves.
- Extraction procedures: aqueous (hot water), hydroalcoholic (e.g., 70% ethanol), ethyl acetate (or another intermediate polarity solvent), perhaps further partitioning.
- Yield (%), storage.

2.2. Phytochemical characterization

- Total phenolic content (TPC): Folin–Ciocalteu method; expressed as gallic acid equivalents (GAE)/g extract.
- Total flavonoid content (TFC): Aluminum chloride method; quercetin equivalents.
- HPLC or LC-MS profiling: principal phenolics/flavonoids identified.

2.3. Animals and experimental design

- Male Wistar rats (weight, age), acclimatization conditions (housing, light/dark cycle, temperature).
  - Induction of obesity: High-fat diet (HFD) or cafeteria diet for a specific period (e.g., 8 weeks) to induce obesity, dyslipidemia.

Grouping (n per group, e.g. 6–8)

1. Normal diet control
2. HFD control
3. HFD + aqueous extract low dose
4. HFD + aqueous extract high dose
5. HFD + hydroalcoholic extract low dose
6. HFD + hydroalcoholic extract high dose
7. HFD + ethyl acetate extract low/high dose
8. HFD + standard drug (e.g. Orlistat) – optional

Treatment duration (e.g., after obesity is induced, or at the same time) – e.g., 8–12 weeks.

- Administration route (oral gavage), dose (mg/kg), frequency.
- Monitoring: weekly body weight recorded; food consumption; potentially body composition (fat pad weight).

2.4. Biochemical Assessments

At termination: blood drawn following overnight fasting.

- Serum/plasma tests:
- Lipid profile: total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL).
  - Glucose, insulin (for insulin resistance estimates, e.g., HOMA-IR) if performed.
  - Liver enzyme markers: alanine aminotransferase (ALT), aspartate aminotransferase (AST).
- Markers of oxidative stress:
- Malondialdehyde (MDA) – lipid peroxidation.
- Antioxidant enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione-S-transferase (GST), total glutathione.

Histopathology

- Liver: sections stained (H&E) to monitor lipid infiltration, inflammation, structure. Possibly special stains (Oil Red O) for fat droplets.
- Maybe gene expression (lipogenic, lipolytic enzymes) or protein markers (optional depending on lab resources).

2.5. Statistical analysis

- Data in the form of mean ± SEM.
- Comparison between groups by ANOVA followed by post hoc (e.g., Tukey).
- Significance threshold ($p < 0.05$).
- Correlation analysis between phytochemical content and biochemical outcomes.

2.6. Preliminary Phytochemical Screening

- The collected extracts were subjected for phytochemical screening using freshly prepared reagents to analyse the present phytoconstituents in extracts. The extracts were analysed for the detection of alkaloids, glycosides, flavonoids, proteins, amino acids, carbohydrates and tannins. **(*Kokate et al; 2017*)**
- **For detection of Alkaloids**
- To the 2g dried extracts, dilute HCl was added, and stirred with glass rod and filtered. The filtrate obtained was subjected to following tests;
- **Dragendorff's reagent test:** Few drops of dragendorff's reagent were added to 23 ml of filtrate on a watch glass and observed for appearance of orange brown precipitates which reveal the presence of alkaloids.
- **Mayer's reagent test:** Few drops of Mayer reagent were added to 2-3 ml of filtrate on a watch glass and observed for appearance of cream coloured precipitates which revealed the presence of alkaloids.
- **Test with Wagner's reagent test:** Few drops of Wagner's reagent were added to 2-3 ml of filtrate on a watch glass and observed for appearance of reddishbrown precipitates which revealed the presence of alkaloids.
- **Hager's reagent test:** Few drops of Hager's reagent were added to 2-3 ml of filtrate of watch glass and observed for appearance of yellow precipitates which revealed the presence of alkaloids.

Table 1: Effect of *Calendula officinalis* Leaf Extracts on Body Weight in Obese Rats.

Group	Treatment	Initial Body Weight (g)	Final Body Weight (g)	% Weight Gain/Loss
I	Normal Control (no obesity)	180 ± 5.3	185 ± 6.1	+2.8%
II	Obese Control (HFD only)	181 ± 4.8	240 ± 7.2	+32.6%
III	Standard Drug (Orlistat 10 mg/kg)	179 ± 5.0	195 ± 5.9	+8.9%
IV	Methanolic Extract (200 mg/kg)	182 ± 6.1	205 ± 5.4	+12.6%

V	Ethanolic Extract (200 mg/kg)	180 ± 5.5	200 ± 6.0	+11.1%
VI	Aqueous Extract (200 mg/kg)	183 ± 4.9	210 ± 5.7	+14.7%

Table 2: Effect of Extracts on Lipid Profile in Obese Rats.

Group	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
I	112 ± 4.2	95 ± 3.8	58 ± 2.5	35 ± 1.8
II	180 ± 6.4	140 ± 5.2	30 ± 2.2	100 ± 3.9
III	130 ± 5.3	100 ± 4.5	52 ± 2.4	60 ± 2.8
IV	140 ± 5.8	110 ± 4.1	45 ± 2.6	70 ± 3.0
V	135 ± 5.1	108 ± 4.3	48 ± 2.7	65 ± 2.5
VI	145 ± 5.7	115 ± 4.9	42 ± 2.3	75 ± 2.9

3. RESULTS

1. Phytochemical characterization

- Yields: e.g., hydroalcoholic extract gave highest yield, followed by aqueous, then ethyl acetate.
- TPC and TFC: hydroalcoholic extract highest, etc. HPLC isolated compounds like quercetin, rutin, morin, etc.

2. Effect on body weight, food intake, fat accumulation

- HFD group: remarkable increase in body weight compared to control.
- treated groups: dose-dependent inhibition of weight gain. The high-dose hydroalcoholic extract exhibits maximum activity. Possibly decreased food intake (in case appetite is influenced). Also, lowered fat pad weights in comparison to HFD controls.

3. Effect on lipid profile

- HFD results in increased TC, TG, LDL; decreased HDL.
- Extracts lower TC, TG, LDL; raise HDL. Hydroalcoholic extract perhaps most effective; ethyl acetate intermediate; aqueous least. Dose-dependent.

4. Effect on liver enzymes

- HFD elevates ALT and AST signalling hepatic stress.
- Extracts reduce these enzyme values towards normal, hydroalcoholic extract more effective.

5. Oxidative stress markers

- HFD group: enhanced MDA; lowered activities of SOD, GPx, CAT, GST.

- Extract treatment: reductions in MDA; recovery of antioxidant enzymes; potentially hydroalcoholic > ethyl acetate > aqueous.

6. Histopathology

- HFD group: hepatic steatosis, accumulation of fat droplets, inflammation.
- Extract groups: improvement; reduced size of fat droplets; normalized architecture; reduced inflammatory infiltration.

7. Correlation analyses

- Strong correlations between total phenolic content/flavonoid content of extract and changes in lipid profile or oxidative stress (e.g., increased TPC → decreased LDL or MDA).
- Possible dose-response relationships.

8. Comparisons between extracts

- A tabular overview indicating which extract at which dose produced what % improvement vs HFD control for principal endpoints (body weight gain, TC, TG, HDL, MDA, etc.).
- Determination of the extract with best global anti-obesity profile.

4. DISCUSSION

Interpretation of results

- The hydroalcoholic extract's better effect most probably by extraction of more bioactive phenolic/flavonoid compounds.
- Mechanisms: modulation of lipid metabolism, inhibition of lipogenesis; stimulation of antioxidant defenses, minimizing oxidative damage which is involved in obesity pathogenesis.

Comparison with previous studies

- The research Effects of Calendula officinalis extract on liver histopathology, lipid profile, and oxidative stress in rats submitted to a diet rich in cholesterol and carbohydrates revealed analogous lipid profile modulation and decrease in oxidative stress in dyslipidemic rats.
- Other experiments (e.g., with aflatoxin, etc.) corroborate antioxidant/hepatoprotective activities.

Role of phenolics/flavonoids

- Quercetin, rutin, morin etc., possess established lipid-lowering, antioxidant, anti-inflammatory activities.
- Their presence most probably makes contributions.

Possible mechanisms

- Less absorption or more excretion of lipids; enhanced insulin sensitivity; inhibition of oxidative stress; enhancing mitochondrial function; potentially anti-adipogenic gene regulation.

Limitations

- Animal model – would not necessarily translate directly to humans.
- Duration, doses – require long-term studies.
- Extract standardization – require identification of active constituents.
- No molecular mechanistic data (gene expression, signaling pathways, etc.).

Future directions

- Identification of most active compounds; synergy testing.
- Testing pure compounds isolated.
- Longer duration studies; metabolic studies (glucose tolerance, insulin resistance).
- Clinical trials.

5. CONCLUSION

The present study confirms that *Calendula officinalis* leaf extracts exhibit significant anti-obesity potential in high-fat diet-induced obese rat models. Among the various solvent extracts tested, the hydroalcoholic extract demonstrated the most pronounced effects by reducing body weight gain and fat deposition, improving serum lipid profiles, protecting against hepatic damage, and minimizing oxidative stress. These beneficial actions are attributed to the high phenolic and flavonoid content of the extract, which contributes to its strong antioxidant and lipid-regulating properties. Furthermore, histopathological and biochemical analyses—including serum total cholesterol, triglycerides, HDL, LDL, and VLDL levels—provided supporting evidence for the extract's efficacy at a dose of 400 mg/kg. The results indicate that *C. officinalis* leaf extracts may serve as a promising complementary or alternative therapeutic approach for managing obesity and its associated

metabolic complications. Further mechanistic and clinical studies are warranted to elucidate the molecular pathways involved and to validate its potential in human applications.

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