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# Microbial decontamination of medicinally important herbals using gamma radiation and their biochemical characterisation

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ABSTRACT

A comprehensive study was carried out to assess the microbiological and biochemical characteristics of four herbals, namely, rose (*Rosa centifolia*), guggul (*Commiphora mukul*), chirata (*Swertia chirayita*), gulvel (*Tinospora cordifolia*) and four herbal formulations rasayan, shatpatryadi, scrub and kashayam. Total aerobic plate count (TAPC) was in the range of 3–7 log cfu/g, whereas, presumptive coliform count in many of these samples was in the range 2–6 log cfu/g. The IMViC (indole, methyl red, Voges-Proskauer, citrate) analysis and molecular characterisation (16S rDNA sequencing) ascertained the presence of *Escherichia coli* in some of the samples. A gamma radiation dose of up to 10 kGy was found to be sufficient for complete microbial decontamination without affecting the bioactive properties of herbal formulations, including antioxidant potential, which was high in rasayan, shatpatryadi, scrub, rose, and guggul. The antioxidant property of these herbals could be attributed to components such as phenolics, flavonoids and colour pigments.



## 1. Introduction

Many herbal products are traditionally being used as medicines and nutraceuticals in different regions of the world. A large proportion (70–80%) of the world population, particularly in developing countries, prefers to use non-conventional medicines, mainly from herbal sources (Soriani, Satomi, & Pinto, 2005). Like traditional Chinese medicines, Indian herbal medicines are also gaining recognition in other regions of the world, including America, Europe and Australia (Frawley, 2007). Herbal medicines are of plant origin; hence they are considered to have fewer side effects and are safe for consumption. However, microbiological contamination of herbs, that could occur either during pre or post harvest processing, is of serious concern (Soriani et al., 2005). Microbial decontamination using ethylene oxide or methyl bromide is prohibited and being phased out in several countries, for health, environment and occupational safety reasons. Also, decontamination, achieved by high temperature treatment, may result in reduced bioactivity of herbal products. Therefore, treatment of herbal products by gamma radiation provides an effective alternative for reducing or eliminating microbial contamination (Farkas, 1988).

Herbal medicines bring relief by different mechanisms of action, including antioxidant properties that help reduce toxicity while enhancing bioefficacy (Govindarajan, Vijayakumar, & Pushpangadan, 2005). The antioxidant property of herbals is mainly conferred by the presence of phenolics and flavonoids (Langley-Evans, 2000). Although known to have clinically significant effects, these herbals still need to be explored further for scientific insights into their exact mechanisms of action and the bioactive principles involved.

The aim of the present work was to study certain commonly used Indian herbal products, namely rose (*Rosa centifolia*), guggul (*Commiphora mukul*), chirata (*Swertia chirayita*) and gulvel (*Tinospora cordifolia*) and herbal formulations (rasayan, shatpatryadi, scrub and kashayam) for characterising their physical, biochemical and microbiological properties. The study was also aimed to standardise process parameters, to achieve microbial decontamination by using gamma radiation.

## 2. Materials and methods

### 2.1. Procurement and extraction of herbal samples

Guggul and chirata powders were procured from a local herbal medicine shop. Rose, gulvel, rasayan, shatpatryadi, scrub and kashayam powders were gifted by Atharva Holistic Healthcare and Foods, Mumbai, Maharashtra, India. For 1% aqueous extract preparation, 0.5 g of herbal powder was homogenised in 50 ml of milli Q water, using a mortar and pestle, vortexed and centrifuged at 10,000*g* for 20 min. The supernatant was collected and filtered through a 0.45 μm filter (Millex-HV, Millipore, Ireland) and stored at 4 °C till the analysis was performed.

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### 2.2. Chemicals

Ascorbic acid, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), Folin–Ciocalteu's phenol reagent, gallic acid, catechin hydrate, aluminium chloride hexahydrate, sodium nitrate, pyrogallol, cetyltrimethylammonium bromide (CTAB), and agarose were procured from Sigma–Aldrich, Inc, St. Louis, MO, USA. Luria–Bertani agar (LA), violet red bile agar (VRBA), tryptone broth, buffered glucose broth (methyl red–Voges-Proskauer medium), Koser citrate, Kovac's indole reagent, O'Meara reagent, methyl red indicator, ferric chloride and potassium ferricyanide were purchased from Himedia Laboratories Ltd., Mumbai, India. Sodium chloride and trichloroacetic acid were purchased from S.D. Fine-Chem. Ltd., Mumbai, India.

### 2.3. Microbiological analyses

#### 2.3.1. Total aerobic plate count (TAPC) and presumptive coliform count

Total aerobic microbial load and presumptive coliform contamination in the herbal samples were determined as described by Soriani et al. (2005). A 5% sample suspension was prepared in 0.85% saline aseptically under a laminar flow. This was serially diluted, plated on a Luria–Bertani agar plate and incubated at 28 ± 2 °C for 48 h to determine the total aerobic plate count (TAPC). Similarly, the presumptive coliforms were enumerated by pour-plating on a violet red bile agar (VRBA) plate and incubating at 35 ± 2 °C for 24 h. The presumptive coliforms were identified as pinkish-red colonies on a VRBA plate.

#### 2.3.2. Biochemical assays for confirmation of Coliform

Presumptive coliform colonies (~12 in numbers) from VRBA plate were randomly picked, streaked and purified on a Luria–Bertani agar (LA) plate. These isolates were analysed using the indole–methyl red–Voges-Proskauer (VP)–citrate (IMViC) test, a confirmatory biochemical test for coliform estimation (US FDA, 2002). The IMViC test includes analysis of indole production in tryptone broth, which is indicated by a bright pink colour formation on the top layer immediately after addition of Kovac's indole reagent. Acid production was tested by the MR (methyl red) test in buffered glucose broth. Positive colonies were indicated by red colour formation on adding methyl red indicator. The VP (Voges-Proskauer) test indicates diacetyl ($CH_3COCOCH_3$) production in buffered glucose broth, which is assayed for pink colour formation upon addition of O'Meara reagent, followed by incubation at ambient temperature (26 ± 2 °C) for 30 min. Citrate utilisation ability is checked in Koser citrate medium.

#### 2.3.3. Molecular characterisation

IMViC positive coliforms were further confirmed by molecular characterisation, using 16S rDNA sequence analysis. Genomic DNA was extracted by the method described earlier (Ausubel et al., 1987). In brief, 5 ml of overnight grown culture was centrifuged at 10,000*g* for 10 min and the pellet was suspended in 475 μl of TE buffer (Tris–HCl, 10 mM; EDTA, 1 mM; pH 8.0). To the suspension, 25 μl of SDS (10%) and 5 μl of proteinase K (10 mg/ml) were added and incubated at 37 °C for 1 h. Then, 90 μl of 5 M NaCl and 75 μl of CTAB/NaCl solution (CTAB, 0.27 M; NaCl, 0.70 M) were added, mixed gently and incubated at 65 °C for 20 min. The mixture was extracted with an equal volume of chloroform:isoamyl alcohol (24:1), centrifuged at 10,000*g* for 10 min and the aqueous phase was collected. This was further extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and centrifuged at 10,000*g* for 10 min. The aqueous phase was again collected and precipitated with isopropanol (0.6 volumes) for 20 min, followed by centrifugation at 15,000*g* for 15 min. The pellet was washed twice with 150 μl of 70% ethanol, air-dried, dissolved in milli Q water and stored at −20 °C. The DNA solution was used as a template for the polymerase chain reaction (PCR).

The PCR mixture was prepared according to the recommendation of the manufacturer. The PCR programme was as follows: initial denaturation step at 94 °C for 3 min, followed by 32 cycles at 94 °C for 45 s, 57 °C for 45 s, and 72 °C for 45 s, followed by a final extension at 72 °C for 10 min. The universal primers, UNI 1 (5′-AAGGAGGTGATCCAGCCGCA-3′) and UNI 2 (5′-AGAGTTTGATCCTGGCTCAG-3′) were used for PCR. The PCR product (~1.4 kb) was extracted from gel using an agarose gel extraction kit (QIAquick, QIAGEN, Germany) and was sequenced. The sequence data were analysed using the BLAST system (http://www.ncbi.nih.gov/BLAST/) developed by the National Centre for Biotechnology Information (NCBI; Bethesda, MD, USA).

### 2.4. Gamma irradiation

Gamma irradiation was carried out in a cobalt-60-based Gamma Chamber (GC-5000, BRIT, Mumbai, India) at the Food Technology Division, Bhabha Atomic Research Centre, Mumbai, India. Samples (3 g) were packed, heat-sealed in a high density polythene (HDPE) bag and irradiated at doses of 1–10 kGy (dose rate 6.5 kGy/h). Treated and control samples were stored at ambient temperature (26 ± 2 °C) until the analyses were carried out.

### 2.5. Spectrophotometric measurements

Spectrophotometric measurements were performed using UV–VIS spectrophotometer (Unicam, Cambridge, UK) at the wavelength mentioned. All the measurements were carried out at least in triplicate.

### 2.6. Physicochemical analyses

#### 2.6.1. General

Samples were analysed for water activity, pH, ash content, electrical conductivity, colour intensity, phenolic, flavonoid and protein contents.

#### 2.6.2. Water activity ($a_w$) and pH measurement

Herb samples (1.5 g) were used for determination of water activity ($a_w$) using a water activity meter which works by the chilled-mirror dew point technique. The instrument displays the water activity of the sample, based on its equilibrium relative humidity (ERH), where $a_w$ and ERH are related as $a_w$ = ERH (in percentage)/100 (AqualabCX2T, Decagon Devices, USA).

The pH of a 1% aqueous extract of samples was measured using a pH analyzer (Li 614, Elico Ltd., Hyderabad, India) calibrated with pH 4.2 and 9.4 buffers.

#### 2.6.3. Ash content and electrical conductivity analysis

The ash content of the samples was determined using a procedure of the Standard 104/1 of the International Association for Cereal Chemistry (ICC, 1993). Briefly, 2 g of sample was placed in a crucible and incubated at 640 °C for 6 h in a muffle furnace. The residue was weighed and the mean value was expressed in g%.

The electrical conductivity was measured using a Conductivity Bridge (type CLOI/02A) for 1% aqueous extract of samples prepared in milli Q water (Fjell, Seibel, & Gerstenkorn, 1996). The measurement of electrical conductivity is based on the determination of the electrical resistance, which is a reciprocal of the electrical conductivity. The readings were expressed in μS/cm.

#### 2.6.4. Colour intensity ($ABS_{450}$) analysis

The colour intensity ($ABS_{450}$) was determined by the method of Bertoncelj, Dobersek, Jamnik, and Golob (2007). Absorbance of 1%

aqueous extract was measured using a UV–VIS spectrophotometer at two wavelengths, 450 and 720 nm. The difference was expressed as milli absorbance units (mAU).

*2.6.5. Phenolic content*

The total phenolic content was determined by the Folin–Ciocalteu colourimetric method (Singleton & Rossi, 1965). Phenols react with phosphomolybdic–phosphotungustic components in the Folin–Ciocalteu reagent and produce a blue coloured complex which is measured at 765 nm. In a separate set of experiments, 25 μl aliquots of 1% aqueous extract of kashayam, chirata and gulvel and 0.5% aqueous extract of rasayan, shapatrayaadi, scrub, rose and guggul were diluted to 800 μl using milli Q water. The mixture was further mixed with 50 μl of 0.2 N Folin–Ciocalteu reagent and incubated at ambient temperature (26 ± 2 °C) for 2 min. A 150 μl aliquot of sodium carbonate solution (0.2 g/ml) was added to the reaction mixture and incubated at the same temperature for 2 h. The absorbance of the reaction mixture was measured and the result was expressed as gallic acid equivalents (mg GAE/g) of the sample.

*2.6.6. Flavonoid content*

The flavonoid content was determined spectrophotometrically by the aluminium chloride colourimetric method (Chen, Lin, & Hsieh, 2007). Aluminium chloride forms pink-coloured complexes with flavonoids which are measured at 510 nm. In a separate set of experiments, a 100 μl aliquot of 1% aqueous extract of samples was mixed with 2.15 ml of milli Q water and 75 μl of $NaNO_2$ (5%). The suspension was mixed with 150 μl of $AlCl_3$ (10%) and incubated at ambient temperature (26 ± 2 °C) for 6 min. Later, 1 ml of NaOH (1 M) was added and the suspension was further incubated for 5 min. The absorbance of the reaction mixture was measured immediately and the result was expressed as catechin equivalents (mg CAE/g) of the sample.

*2.6.7. Protein content*

The total protein content was determined by Bradford's method, using a protein estimation kit (Bangalore Genei, Bangalore, India), and bovine serum albumin (BSA) as a standard. Proteins bind to Coomassie brilliant blue G 250 dye and form a complex whose extinction coefficient is greater (λ max = 595 nm) than that of the free dye (λ max = 465 nm). A 200 μl aliquot of 1% aqueous extract was used for determining the protein content. The absorbance was measured at 595 nm using a spectrophotometer. The protein content was expressed in milligrams per gram (mg/g) of sample.

*2.7. Antioxidant assays*

*2.7.1. Antioxidant content*

The antioxidant content, in terms of ascorbic acid equivalent antioxidant content (AEAC), was determined by the method described earlier (Meda, Lamien, Romito, Millogo, & Nacoulma, 2005). The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) is a stable free radical and converts to 1,1-diphenyl-2-picryl-hydrazine (reduced form) in the presence of hydrogen-donating substance. The DPPH solution (0.4 mM) was prepared in absolute ethanol. In a separate set of experiments, a 100 μl aliquot of 1% aqueous extract of kashayam, chirata and gulvel and 0.33% aqueous extract of rasayan, shatpatryadi, scrub, rose and guggul were added to 400 μl of ethanol (70%). Further, 500 μl of DPPH solution was mixed thoroughly and allowed to stand for 15 min at ambient temperature (26 ± 2 °C). The absorbance was measured at 517 nm. The antioxidant content was expressed as milligrammes of ascorbic acid equivalent antioxidant content (AEAC) per gramme of sample.

*2.7.2. Reducing power*

The reducing power of the aqueous extracts was determined using the potassium ferricyanide reduction method (Oyaizu, 1986). The assay is based on the formation of Perl's Prussian Blue, due to the reduction of potassium ferricyanide, resulting in an increase in the absorbance at 700 nm. In brief, a 100 μl aliquot of 1% aqueous extract was mixed with 250 μl of phosphate buffer (0.2 M, pH 6.6) and 250 μl of potassium ferricyanide (1%). The suspension was incubated at 50 °C for 20 min. Later, 250 μl of 10% trichloroacetic acid was added and mixed thoroughly. The suspension was centrifuged at 3000*g* for 10 min. A 250 μl aliquot of the supernatant was mixed with 250 μl of milli Q water and 50 μl of $FeCl_3$ (0.1%). The suspension was diluted by adding 1.1 ml of milli Q water and the absorbance was measured.

*2.7.3. Superoxide scavenging activity*

The superoxide scavenging activity was determined using pyrogallol (An et al., 2004). The pyrogallol undergoes superoxide anion-dependent autoxidation at alkaline pH (7.9–9.1) and hence, this property was used to test the ability of any test compound to inhibit pyrogallol autoxidation. The reaction mixture containing 2 ml of aqueous extract (1%), 2.6 ml of TE buffer (Tris–HCl, 100 mM; EDTA, 20 mM; pH 8.5), 1 ml of milli Q water and 0.4 ml of pyrogallol (10 mM) was incubated at 25 °C for 10 min and the absorbance was measured at 420 nm. The superoxide scavenging activity (%) was calculated by using the following equation:

$$\text{Superoxide scavenging activity } (\%) = [1 - \{(T_1 - B_2)/B_1\}] \times 100$$

where $T_1$, $B_1$, and $B_2$ denote the absorbances of the sample, sample blank and pyrogallol blank, respectively.

## 3. Results and discussion

*3.1. General*

The herbals analysed in the present studies are traditionally being used for their therapeutic properties. The rose petal extract has been reported to possess anti-inflammatory and analgesic effects and is also used for skin care (Choi & Hwang, 2003). The guggul gum extract has been found to be beneficial in atherosclerosis and hypercholesterolemia (Singh, Mishra, et al., 2003). The medicinal uses of gulvel are based on hepatoprotective, immunomodulatory, and anti-inflammatory properties (Singh, Pandey, et al., 2003). The extract of chirata has been found to be antihelmintic, hypoglycaemic and antipyretic (Joshi & Dhawan, 2005). Herbal formulations used in these studies include several constituents in varying proportions, probably, based upon their clinical requirement. Among formulations studied, all are used as internal medicines, except scrub, which is used only for the external applications. Table 1 displays the list of formulations studied, their clinical significance and constituents.

*3.2. Microbiological analyses*

The microbiological profile of the samples is shown in Table 2. Total aerobic plate count (TAPC) was in the range 3–7 log cfu/g. Presumptive coliform count in many of the samples ranged from 2 to 6 log cfu/g. Microbiological load was found to be comparatively higher in gulvel (6.9 log cfu/g) and chirata (6.8 log cfu/g) amongst the herbals and formulations analysed. The presumptive coliform count was highest in gulvel (5.6 log cfu/g), followed by chirata (4.8 log cfu/g), shatpatryadi (3.9 log cfu/g) and rose (2.3 log cfu/g), whereas, coliforms were not detected in kashayam, scrub, rasayan or guggul. Similar microbial contamination levels have been reported in *Ginkgo biloba*, *Paullinia cupana* and certain

**Table 1**
Herbal formulations, their medicinal uses and constituents.

| Formulations | Medicinal uses | Plant material | Plant-part used | Quantity (g%) |
|---|---|---|---|---|
| 1. Rasayan | Anti ageing, reduces debility | *Punica granatum* | Seed | 15 |
| | | *Terminalia chebula* | Fruit | 10 |
| | | *Withania somnifera* | Root | 10 |
| | | *Phyllanthus emblica* | Fruit | 10 |
| | | *Tribulus terrestris* | Whole plant | 10 |
| | | *Centella asiatica* | Leaf | 10 |
| | | *Terminalia arjuna* | Bark | 10 |
| | | *Asparagus racemosus* | Root | 7.5 |
| | | *Hemidesmus indicus* | Root | 7.5 |
| | | *Tinospora cordifolia* | Stem | 5 |
| | | *Glycerrhiza glabra* | Rhizome | 5 |
| 2.Shatpatryadi[a] | Reduces acidity, gastritis, dyspepsia | *Rosa centifolia* | Bud | 50 |
| | | *Mallotus philippensis* | Petal | 5 |
| | | *Cyperus rotundus* | Rhizome | 2.5 |
| | | *Cuminum cyminum* | Seed | 2.5 |
| | | *Santalum album* | Heart wood | 2.5 |
| | | *Elettaria cardamomum* | Seed | 2.5 |
| | | *Foeniculum vulgare* | Seed | 2.5 |
| | | *Grewia asiatica* | Fruit | 2.5 |
| | | *Piper cubeba* | Fruit | 2.5 |
| | | *Tinospora cordifolia* | Stem | 2.5 |
| | | *Papaver somniferum* | Seed | 2.5 |
| | | *Plantago ovata* | Seed husk | 2.5 |
| | | *Tribulus terrestris* | Whole plant | 2.5 |
| | | *Cinnamomum tamala* | Leaf | 2.5 |
| | | *Cinnamomum tamala* | Bark | 2.5 |
| | | *Mesua ferrea* | Stamen | 2.5 |
| | | *Hemidesmus indicus* | Root | 2.5 |
| | | *Acacia catechu* | Bark | 2.5 |
| | | *Prunus amygdalus* | Seed | 2.5 |
| 3. Kashayam | Cough, cold, rhinitis | *Glycerrhiza glabra* | Rhizome | 50 |
| | | *Zingiber officinale* | Rhizome | 25 |
| | | *Adhatoda vasaka* | Leaf | 20 |
| | | *Piper longum* | Fruit | 5 |
| | | *Cinnamomum tamala* | Bark | 5 |
| | | *Piper nigrum* | Fruit | 2.5 |
| | | *Elettaria cardamomum* | Seed | 2.5 |
| | | *Curcuma longa* | Rhizome | 2.5 |
| 4. Scrub | Skin care | *Phyllanthus emblica* | Fruit | 20 |
| | | *Curcuma amada* | Rhizome | 20 |
| | | *Cyperus rotundus* | Rhizome | 20 |
| | | *Vetiver zizzoides* | Root | 10 |
| | | *Centella asiatica* | Leaf | 10 |
| | | *Rubia cordifolia* | Stem | 5 |
| | | *Hedychium spicatum* | Rhizome | 5 |
| | | *Santalum album* | Heart wood | 5 |
| | | *Citrus aurantium* | Bark | 2.5 |

[a] Shatpatryadi also contains natural calcium oxide (5%) from conch shell.

**Table 2**
Microbial count (log cfu/g) in herbals and formulations and its decontamination using gamma radiation.

| Dose (kGy) | Rose | | Guggul | | Chirata | | Gulvel | | Rasayan | | Shatpatryadi | | Kashayam | | Scrub | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TAPC[a] | PC[b] | TAPC | PC | TAPC | PC | TAPC | PC | TAPC | PC | TAPC | PC | TAPC | PC | TAPC | PC |
| 0 | 5.0 ± 0.1 | 2.3 ± 0.2 | 4.1 ± 0.1 | ND[c] | 6.8 ± 0.1 | 4.8 ± 0.4 | 6.9 ± 0.1 | 5.6 ± 0.02 | 5.7 ± 0.2 | ND | 4.9 ± 0.03 | 3.9 ± 0.1 | 4.3 ± 0.1 | ND | 6.1 ± 0.6 | ND |
| 1.0 | 4.5 ± 0.1 | ND | 3.2 ± 0.03 | ND | 6.1 ± 0.04 | 4.1 ± 0.1 | 5.8 ± 0.4 | 4.7 ± 0.1 | 5.5 ± 0.1 | ND | 4.4 ± 0.1 | ND | 3.9 ± 0.2 | ND | 5.3 ± 0.1 | ND |
| 2.5 | 4.2 ± 0.2 | ND | 2.5 ± 0.2 | ND | 5.3 ± 0.1 | 3.6 ± 0.1 | 3.7 ± 0.1 | 3.0 ± 0.3 | 4.4 ± 0.1 | ND | 4.2 ± 0.2 | ND | 3.2 ± 0.3 | ND | 4.8 ± 0.01 | ND |
| 5.0 | 2.9 ± 0.1 | ND | ND | ND | 3.9 ± 0.2 | ND | ND | ND | 3.6 ± 0.2 | ND | 2.6 ± 0.4 | ND | ND | ND | 2.9 ± 0.2 | ND |
| 7.5 | ND | ND | ND | ND | 3.1 ± 0.2 | ND | ND | ND | 2.6 ± 0.2 | ND | ND | ND | ND | ND | ND | ND |
| 10 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |

[a] TAPC = total aerobic plate count.
[b] PC = presumptive coliform.
[c] ND = not detected.

other botanical raw materials where TAPC and presumptive coliform were 3–8 and 2–3 log cfu/g, respectively (Soriani et al., 2005).

To ascertain the nature of these presumptive coliforms, biochemical characterisation was carried out using IMViC analysis. *Escherichia coli* can be identified as two different biotypes, type I (++−−) and type II (−+−−), by IMViC tests (US FDA, 2002). Further, 16S rDNA sequence analysis was performed to confirm the molecular identity of the randomly picked IMViC-positive *E. coli*

biotypes. Analysis showed that, among these IMViC-positive *E. coli*, the 16S rDNA sequence of the type I biotype closely matched the *E. coli* sequence. Among the eight samples studied, *E. coli* contamination was found in three herbs and formulations, namely gulvel, chirata and shatpatryadi, which correspond to 37.5% of the samples analysed. Molecular characterisation indicated the presence of confirmed *E. coli* in gulvel, chirata and shatpatryadi, at levels of 16%, 55% and 28%, respectively, in the randomly picked population of IMViC-positive *E. coli* isolates. According to United Nations Industrial Development Organization (UNIDO, 1984), the permissible levels of microbial contamination in herbal raw materials are as follows: total aerobic bacteria <4 log cfu/g, *Enterobacter* and other Gram-negative organisms <2 log cfu/g and *E. coli* nil. As observed in this study most of the herbal products analysed were found to have microbial loads above the permissible level (Table 2). Therefore, these findings emphasise the need for microbial decontamination of the raw herbal samples for ensuring hygiene and health safety before their clinical uses.

### 3.3. Microbial decontamination using gamma radiation

Radiation treatment was explored as a possible means to achieve microbial decontamination of the herbal products because it has been established as a safe process which, in general, does not affect the intrinsic physicochemical properties of the products (Diehl, 1995). The treatment with gamma radiation has been approved by international bodies, namely FAO, IAEA and WHO. Their joint expert committee report, published in 1980, recommended irradiation as a safe process to achieve hygienization without causing any nutritional, microbiological or toxicological concerns (Diehl, 1995). Hence, herbal products were gamma-irradiated doses ranging from 1 to 10 kGy. A complete microbial decontamination was achieved at the 10 kGy dose in most of the samples (Table 2). In gulvel and chirata, where coliform counts were higher, a 5 kGy dose was required for complete elimination of coliforms. However, for rose and shatpatryadi, 1 kGy was found to be sufficient for coliform elimination. These results are in agreement with the reports by Soriani et al. (2005) on *G. biloba* and *P. cupana* and reports by Farkas (1988) on dry food ingredients. They achieved a TAPC level below 10 cfu/g at 11.4 kGy and presumptive coliform count almost nil at 5 kGy. Thus, radiation treatment was found to be an effective technique for decontamination of herbs and formulations, ensuring their hygiene and safety.

### 3.4. Physicochemical analyses

#### 3.4.1. General

To characterise the physical nature of these herbals, parameters such as water activity ($a_w$), pH, ash content, electrical conductivity and colour intensity ($ABS_{450}$) were studied whereas, for biochemical characterisation, phenolic, flavonoid and protein contents were analysed.

#### 3.4.2. Water activity ($a_w$) and pH

Herbal products and formulations, being dry products, were found to have low levels of water activity, which ranged from 0.52 to 0.57 (Table 3). Such a low water activity provides longer storage life because the absolute limit for microbial growth is >0.6 (Adam & Moss, 1996). However, it does not ensure the hygiene and safety of herbal products which is a matter of health concern to consumers. Depending on the plant sources, these herbals and formulations were also found to have variations in their acidities. The pH of the samples was found to vary from 3.6 to 6.2 (Table 3). Guggul (pH 3.6), scrub (pH 3.6) and rasayan (pH 3.8) were found to be the most acidic among the herbs and formulations analysed.

#### 3.4.3. Ash content and electrical conductivity

The ash content was in the range 10.3–14.5% for most of the herbals studied, except gulvel, where ash content was 3.5% (Table 3). Ash is the quantity of mineral matter in a sample and there is a linear correlation between the ash content and electrical conductivity (Fjell et al., 1996; ICC Standard 104/1, 1993). The electrical conductivity for most of the samples was $0.03–0.07 \times 10^{-2}$ μS/cm. In gulvel, electrical conductivity was lowest among the samples ($0.003 \times 10^{-2}$ μS/cm) (Table 3). In the present study, a strong correlation ('*r*' = 0.71) was observed between ash content and electrical conductivity of the herbal samples.

#### 3.4.4. Colour intensity ($ABS_{450}$)

Among the samples analysed, the colour intensity ($ABS_{450}$) was highest in chirata (662 mAU) and lowest in gulvel (36 mAU). For other samples, the $ABS_{450}$ was 207–572 mAU (Table 3). A study conducted on honeys of different botanical origin were found to have $ABS_{450}$ ranging from 70–495 (Bertoncelj et al., 2007).

#### 3.4.5. Phenolic content

A large variation in phenolic content was observed in different herbals and formulations (Table 3). Rose was found to have a high amount (104 mg GAE/g) of phenolics. Guggul and three formulations, namely, rasayan, shatpatryadi and scrub, were found have moderate amounts of phenolics (35–51 mg GAE/g). However, phenolic content was low in kashayam and chirata (6 mg GAE/g and 8 mg GAE/g, respectively). Surprisingly, in gulvel, phenolic content was almost negligible. A wide variation in total phenolic contents (2–51 mg GAE/g) has been reported in the aqueous extract of 30 Chinese medicinal plants (Wong, Li, Cheng, & Chen, 2006). In another study with 12 rose cultivars, the total phenolic content of rose petal ranged from 50–119 mg GAE/g (Vinokur et al., 2006).

#### 3.4.6. Flavonoid content

In comparison to the total phenolics, a smaller variation was observed in the flavonoid contents of these herbals and formulations (Table 3). In the majority of the cases, it ranged from 6–11 mg CAE/g, except for kashayam (2 mg CAE/g) and gulvel (0.3 mg CAE/g).

**Table 3**
Physicochemical properties of herbals and formulations.

| Samples | Water activity ($a_w$) | pH | Ash content (g%) | E. C[a] (μS/cm) X $10^{-2}$ | Colour intensity ($ABS_{450}$) (mAU) | Phenolics (mg GAE/g) | Flavonoids (mg CAE/g) | Proteins (mg/g) |
|---|---|---|---|---|---|---|---|---|
| Rose | 0.54 ± 0.00 | 5.1 ± 0.0 | 10.4 ± 0.2 | 0.05 ± 0.03 | 392 ± 31 | 104 ± 7 | 11 ± 1 | 86 ± 8 |
| Guggul | 0.56 ± 0.01 | 3.6 ± 0.0 | 14.5 ± 0.3 | 0.06 ± 0.01 | 402 ± 17 | 35 ± 4 | 7 ± 0.2 | 7 ± 1 |
| Chirata | 0.54 ± 0.02 | 6.1 ± 0.2 | 13.0 ± 0.1 | 0.04 ± 0.02 | 662 ± 55 | 8 ± 3 | 6 ± 1 | 19 ± 1 |
| Gulvel | 0.56 ± 0.00 | 5.8 ± 0.1 | 3.5 ± 0.1 | 0.003 ± 0.00 | 36 ± 08 | 0.1 ± 0.0 | 0.3 ± 0.1 | 3 ± 1 |
| Rasayan | 0.52 ± 0.00 | 3.8 ± 0.0 | 12.3 ± 0.2 | 0.07 ± 0.03 | 496 ± 43 | 51 ± 2 | 7 ± 0.3 | 24 ± 1 |
| Shatpatryadi | 0.55 ± 0.01 | 5.4 ± 0.0 | 12.7 ± 0.1 | 0.03 ± 0.00 | 248 ± 13 | 40 ± 6 | 7 ± 0.1 | 14 ± 0.4 |
| Kashayam | 0.57 ± 0.01 | 5.2 ± 0.2 | 11.0 ± 0.1 | 0.04 ± 0.02 | 207 ± 12 | 6 ± 1 | 2 ± 0.4 | 6 ± 1 |
| Scrub | 0.52 ± 0.00 | 3.6 ± 0.1 | 10.3 ± 0.3 | 0.06 ± 0.00 | 572 ± 21 | 46 ± 2 | 8 ± 0.2 | 18 ± 3 |

[a] E.C = electrical conductivity.

Flavonoid contents ranging from 2.1–4.9 CAE/g were reported in another study with 17 different herbals. In the same study, rose (*Rosa rubiginosa*) hips were found to contain 4 mg CAE/g of flavonoids which was comparatively lower than our observation with rose petal (11 mg CAE/g) (Yoo, Lee, Lee, Moon, & Lee, 2008).

*3.4.7. Protein content*

The total protein content in herbals was observed to be similar to the total phenolic content (Table 3). Rose was found to have a high amount of proteins (86 mg/g). A moderate amount of protein (14–24 mg/g) was found in chirata, rasayan, shatpatryadi and scrub, whereas, guggul, gulvel and kashayam have smaller amounts of protein (3–7 mg/g). The crude protein content has been reported to be in the range 46–220 mg/g for some of the spices and herbs (Achinewhu, Ogbanna, & Hart, 1995).

None of these physicochemical properties were affected by gamma radiation treatment at the dose of 10 kGy ($p < 0.01$) (data not shown).

*3.5. Antioxidant potential*

*3.5.1. General*

Several therapeutic uses of the herbal products are known to be positively correlated with their antioxidant properties as many health disorders have been reported to be free radical-mediated (Govindarajan et al., 2005). Therefore, these herbals and formulations were studied for their antioxidant properties, namely ascorbic acid equivalent antioxidant content (AEAC), reducing power and superoxide scavenging activities and the responsible factors, such as phenolic, flavonoid and protein contents, as described above. Antioxidants scavenge free radicals by getting oxidised themselves. However, the resulting antioxidant radical does not initiate another free radical formation due to delocalization of the radical electron and combines with another radical species to form stable products.

*3.5.2. Ascorbic acid equivalent antioxidant content (AEAC)*

Fig. 1 displays the AEAC (mg/g) of the herbals and formulations. Most of the herbal products and formulations were found to have a high level of AEAC (~28 mg/g), except chirata, kashayam and gulvel. The AEAC was smaller in chirata (6 mg/g) and kashayam (2 mg/g). AEAC was not detected in gulvel. Earlier studies with 26 ginger species reported AEAC in the range 0.3–23 mg/g (Chan et al., 2008).

*3.5.3. Reducing power*

As displayed in Fig. 2, rose, guggul, rasayan and scrub had high levels of ferric reducing power (0.70–0.78). In shatpatryadi, reducing power was relatively low (0.53). Chirata and kashayam were found to have much less reducing power (0.183 and 0.073, respectively), whereas, gulvel had almost negligible reducing power. Similar findings were reported in some other selected nutraceutical herbs (Chen et al., 2007).

*3.5.4. Superoxide scavenging activity*

The superoxide scavenging activity is shown in Fig. 3. The maximum superoxide scavenging activity was found in rasayan (31%), followed by rose (24%). Guggul, chirata, shatpatryadi and gulvel were found to have moderate levels of superoxide scavenging activity (10–12%). Kashayam (1.5%) had very low activity whereas scrub did not have this activity at all. An earlier study, conducted

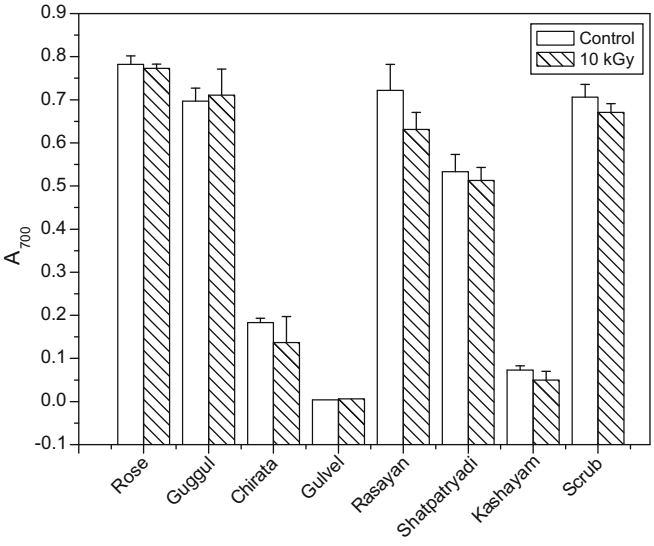


**Fig. 2.** Reducing power of control and irradiated (10 kGy) herbal samples.

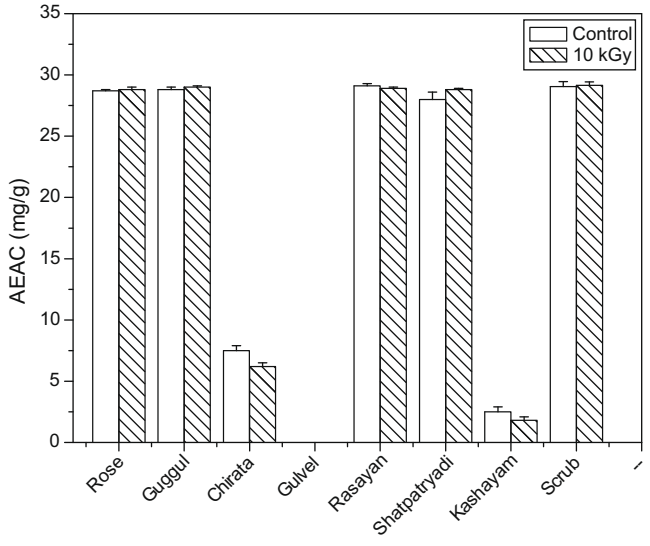


**Fig. 1.** Ascorbic acid equivalent antioxidant content (AEAC) in control and irradiated (10 kGy) herbal samples.

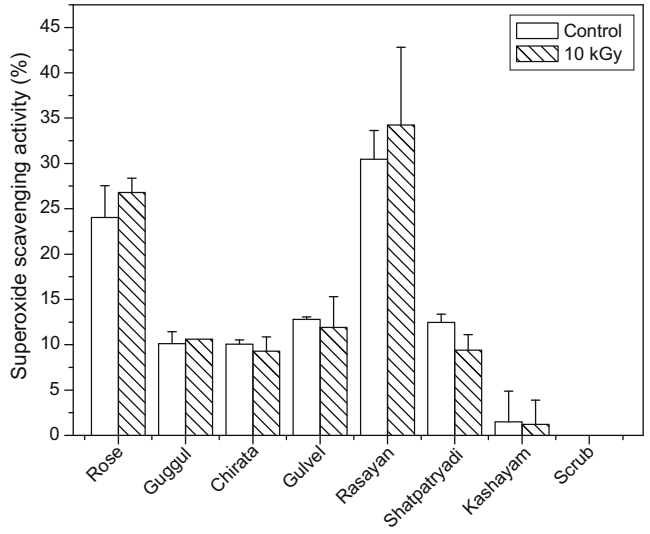


**Fig. 3.** Superoxide scavenging activity in control and irradiated (10 kGy) herbal samples.

on some of the traditional Chinese medicines, showed that herbs vary in their superoxide scavenging activities and sometimes herbs could also produce superoxide (Lin, Chen, & Hew, 1995). The major components of gulvel include alkaloids, glycosides, diterpenoid lactones and steroids (Singh, Pandey, et al., 2003). A moderate superoxide scavenging activity in this herb may be attributed to these component/s since phenolic or flavonoid content was found to be negligible (Gulcin, Mashvildadze, Gepdiremen, & Elias, 2006; Kaji et al., 2009).

The AEAC, reducing power and superoxide scavenging activity were not found to be affected by gamma radiation treatment at 10 kGy ($p < 0.01$) (Figs. 1–3).

Thus, these observations indicated that rose has a high level of AEAC, reducing power and superoxide scavenging activity. A similar trend was observed for guggul, except that its superoxide scavenging activity was relatively smaller. All these activities were found to be comparatively low in chirata. In gulvel, AEAC and reducing power were almost negligible; however, superoxide scavenging activity was significantly higher and comparable to guggul and chirata. As far as formulations are concerned, all these activities were higher in rasayan. In shatpatryadi, AEAC and reducing power were high but superoxide scavenging activity was less. In the case of scrub, AEAC and reducing power were high, whereas superoxide scavenging activity was absent. In kashayam, all of these activities were at low levels. The variation in the levels of these activities in different herbal products and formulations indicates their possible mechanisms of action for scavenging of free radicals, ultimately leading to their pharmacological applications.

### 3.6. Correlation among biochemical parameters

There was a significant correlation between phenol and antioxidant potential of the samples ('*r*' = 0.78 with AEAC and '*r*' = 0.84 with reducing power). An almost similar correlation ('*r*' = 0.79) was observed between the antioxidant activity and total phenolic content of 30 Chinese medicinal plants (Wong et al., 2006). However, the correlation value was comparatively higher for the flavonoids ('*r*' = 0.873 with AEAC and '*r*' = 0.907 with reducing power). As flavonoids are a specialised group of the phenols, having efficient antioxidant ability, this may be the probable reason for their higher correlation values (Langley-Evans, 2000). The correlation between protein content and antioxidant activities was found to be low ('*r*' = 0.41 with AEAC, '*r*' = 0.52 with reducing power). The protein owes its antioxidant activity to constituent amino acids, mainly the aromatic and basic ones. However, the proper spatial position of the amino acids is a very important factor for governing the antioxidant activity of proteins (Rajapakse, Mendis, Jung, Je, & Kim, 2005). A strong positive correlation was observed between AEAC and reducing power ('*r*' = 0.98). In addition, the colour intensity ($ABS_{450}$) was found to have good correlation values ('*r*' = 0.82 and '*r*' = 0.87 with AEAC and reducing power, respectively) for all the tested samples except chirata. The $ABS_{450}$ was also found to have a significant correlation with flavonoids ('*r*' = 0.80). The $ABS_{450}$ is related to pigments (carotenoids, flavonoids) with antioxidant properties (Frankel, Robinson, & Berenbaum, 1998). The correlation between superoxide scavenging activity and other antioxidant parameters was found to be low ('*r*' = 0.14 to 0.54). A wide variability has been reported in other herbals, also with respect to superoxide scavenging activity, probably due to the nature of their constituents (Lin et al., 1995). The significant correlation of colour intensity with AEAC, reducing power or flavonoids, indicated the possible role of colour pigments in the antioxidant activities of these herbs. Thus the study showed that the overall antioxidant property of the samples could be attributed to the synergistic effects of the phenolics, flavonoids and colour pigments.

## 4. Conclusion

A radiation dose up to 10 kGy was found to be sufficient to ensure microbiological safety of the herbals and formulations without affecting their biochemical characteristics. The results also indicated that some of these medicinal herbals and formulations are very rich sources of antioxidants.

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