EFFECT OF GAMMA RADIATION AND DIFFERENT PACKINGS USED FOR STORAGE OF THE DRUG BAHERA FRUIT (TERMINALIA BELERICA ROXB) ON MICROFLORA POPULATION (FUNGI, PATHOGENIC BACTERIAL SP. & SPC) AND ON THE PHYTOCONSTITUENTS (PROTEIN AND TANNIN)

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ABSTRACT: The sample was collected from their natural habitat (Shivalik of Hardwar). Four type of packings were used for storage. These included polythene, cloth, paper and gunny bag. Unpacked sample was used as control. The study was conducted for a period of 24 months (Jan 1990 to Dec 1991). The drug sample is subjected to the following study.

Periodical changes in the microflora population (Fungi, Total Bacterial count (spc) and Pathogenic bacterial species) and on the important phyto constituents (Protein & Tannin of the drug collected from shivalik of Hardwar) during storage under the effect of gamma radiation and different packings (cloth, paper, polythene and Gunny Bag) used.

INTRODUCTION:

The use of plant as drug depends on is medicinal potential, medicinal potential of the plant based medicine depends on its active constituents of the drug which are effective against specific ailments. From the time of collection of the drug to their storage and production of medicines, chances of deterioration in their quality are quite frequent, due obviously to the action of microorganism growing on the drug. This results in a decline in the efficacy of drug. This is an important factor which deserves our attention. It is necessary that drugs are subjected to close screening for their phytochemicals and micro-biological characters prior to their use by pharmacies for producing medicine.

MATERIALS:

The drug sample was collected from field, cut in small pieces, dried and ten stored in different packings (Cloth, Paper, Polythene and Gunny bag). Unpacked sample was used as control.

METHODS:

1. FOR TOTAL BACTERIAL COUNT: Nutrient agarmedia was used for total bacterial count. Plates were incubated a 35oC -37oC for 48 hours. After 48 ours the colonies developed were counted and average for five petridishes was calculated in number of colonies/ml (1)
2. FOR FUNGI: Sabouraud agar, potato dextrose agar and Rose Bengal agar media were used for isolation and identification of fungal species, plates were incubated in BOD incubator at 20°C to 25°C for 72 hours. Fungal colonies developed on the plates was identified according to (2,3 & 4).

3. FOR BACTERIAL PATHOGENS:

(i) Escherichia coli: Nutrient broth, Mac conkeys broth and peptone water were used, finally confirmed by gram’s stain and Indole test(1).

(ii) Salmonella: Nutrient broth, salenite –F- broth, tetra thionate broth, Desoxycholate citrate agar, Bismuth sulphita agar brilliant green agar, triple sugar iron agar and Urea broth were used. Finally confirmed by gram’s stain (1).

(iii) Staphylococcus aureus – Fluid soyabean casein digest medium, manitol salt agar and vogal Johnson agar medium were used. Finally confirmed by Gram’s stain & coagulase test (1)

(iv) Pseudomonas: Cetrimide broth and cetrimide agar were used. Finally confirmed by Gram’s stain and oxides test (1).

4. QUANTITATIVE ESTIMATION OF THE PHYTOCONSTITUENTS:

Estimation of Tannin: Weighted accurately 1 gm of drug powder and was mixed thoroughly with 1 gm of calcium carbonate. The mixture was subjected to soxhlet extraction with ethanol for half hour. The ethanol extract was mixed with 25 ml of water and ethanol was distilled off, from the extract tannin were removed by the addition of 4ml of 10% solution of lead acetate. After preparation in all the aqueous portion yellow coloured precipitate were filtered trough sintered lead acetate. Dried in oven at 100°C, weighted and calculated the total tannin (9).

Estimation of Protein: Weighted accurately 2 gm of drug powder into 200ml long necked flask and 3gm of nitrogen free mercuric oxide and 20ml of nitrogen free sulphuric acid heat the mixture over a small flame until colourless ad boil gently for a further two ours. Cool dilute to about 75ml with water and added a piece of granulated inc and a solution contained 1.5 gm of sodium hydroxide per ml of sulphuric acid used and 2 gm of sodium thiosulphate in 25 ml of water, Ensure that before distillation, the mixture is strongly alkaline by increasing the quantity of the sodium hydroxide immediately connected to the flask to a distillation apparatus, mix the contents distill the liberated ammonia into 50.0ml of 0.1 N sulphuric acid and titrated substance being examined the difference between the titrations represents the ammonia liberated by the substance being examined. Each ml of 0.1 N sulphuric acid is equivalent to 0.00101 gms of nitrogen. (actor of protein-625mg, Factor of nitrogen 1.101 mg) (1).

RESULT AND DISCUSSION

During the course of the present stud a total of 33 species of fungi were recorded the fungal species belonging to 12 genera (Aspergillus, Botrytis, curvularia, fusarium, Gilmaniella, moniella, Mucor, Penicillium, paccelomyces, Rhizopus, Theilavia and Zygosporium); 3 class (Phycomycetes, Ascomycetes, Imperfection) and 6 families (Dematiaceae, Eurotiacea, Moniaceae, Tuberculiacae) were recorded.
The total number of fungal species recorded during the study period was control (27), cloth (28), Polythene (15), paper (131) and gunny bag (33). Treated sample was found free from mgeolflora throughout the study period.

A total of 3 pathogenic bacterial species belonging to 3 genera (Eschirichia, staphylococcus, Salmonella), 2 section (gram-ve facultatively anaerobic rods & gram+ ve cocci) and 2 families (Enterobacteriaceae, Micrococcaceae) were recorded on cloth, paper and gunny bag packed sample, polythene packed and treated was recorded sample was found free from pathogenic bacterial species.

SPC value ranged was recorded 1.6 x10¹ to 10¹ in control, 5.4 x10¹ in cloth, 2x 10¹ to 12 x 10¹ in polythene 10x510¹ in paper 14.5x10¹ to 83x10¹ in gunny bag. Nil count was recorded throughout the study period in treated sample (Gamma radiation) (Table 1)

Studies in respect to biodeterioration of phytoconstituents during the storage sowed that percentage of protein decreased from 19.19 to 11.62 in control, 19.19 to 10.60 in cloth, 19.19 to 15.41 in polythene, 19.19 to 10.01 in paper, 19.19 to 8.12 in gunny bag and 19.19 to 19.01 in treated (Gamma radiation ) (Table 2 & Fig 1)

Percentage of tannin after 4 months was 23.0, 2.665, 23.1, 22.94, 22.90 and after 24 months it was 22.82, 22.78, 22.94, 22.74, 22.70 under control, cloth polythene paper and gunny bag respectively. (Table 2,3 & Fig 1,2)

In Bahera fruit loss of protein percentage in first 12 months was recorded as 33.19, 37.47, 12.92, 10.07 & 47.11 and in 2nd 12 months it was 6.226, 7.29, 6.78, 7.77 and 10.58. Loss of tannin percentage in first 12 months was 1.64, 1.76, 1.12, 1.85, 1.98 and in 2nd 12 months it was 0.17, 0.22, 0.17, 0.30 and 0.34 respectively under control, cloth, polythene, paper and gunny bag.

Studies in respect of periodical changes in fungal population of the drug and formulation under different packing showed definite trend which was similar in all cases except polythene packing, the number of fungal species was found maximum in Aug 1990. Thereafter a decline in their number was observed which continued upto April 1991. a sudden spurt in the number of fungal species during this period could be attributed to increase humidity. The polythene packings showed a different trend from the above have no spurt in the number of fungal species was observed during Aug 1991 under polythene packings, since polythene impervious to moisture these results were only expected (Table 1).

The species which appeared to be the most common and which was found throughout the study period was a spergillus niger. The preponderance of aspergilli on the drugs under study is supported by (8) who found that aspergilli was most frequent.
contaminant of Amla Bahera and harar fruits, according to (10) medicinal plants undergo drastic chemical changes from field to factor due to microbial action and fungi play most vital role in deteriorating drug plants at various stages.

Gilmaniella, paecelomyces, theilavia and botrytis were recorded during the first 12 months thereafter these fungal general were generally not recorded.

The number of pathogenic bacterial species were recorded in control during April 1990 to April 1991 in all packings except unpacked in unpacked pathogenic bacterial species were recorded during Aug 1990 to Dec 1990 the treated & polythene packed samples ere found free from pathogenic bacterial species through out t study period. (Table 1)

Staphylococcus aureus, salmonella and Escherichia coli were recorded during first 16 months thereafter these were not recorded. These species are pathogenic and causes serious diseases like ptyphoid haemolysis, food poisioning, salmonellases etc. Thus there is every possibility tat during collection drying and storage in pharmacies, houses or in factories the drugs get contaminated with various microflora which a not only deteriorate the important phytoconstituents but ma also affect the health of persons adversely.

The spc value was recorded maximum in Aug 1991 in all packings. Thereafter decreased upto Dec 1991 except unpacked and polythene in unpacked and polythene, SPc value were recorded during April 1990 to Aug 1991 and nil count was found in Dec 1991 . packing wise minimum count was recorded in polythene packing. Polythene packing is much more effective in controlling the spc value, since results of polythene packings were only expected treated sample was found free fro Spc throughout the stud period (Table 1).

Loss of the phytoconstituent was more in first 12 months while it was very little in 2nd 12th months. These seems to be definite co- relation between the loss of the phytoconstituents and the fungal population infesting the drugs. Phytoconstituents decreased rapidly in first 12 months because in this period load of microflora was observed high while in the second 12 months due to the decline in microflora, phytoconstituents deterioration slowed down (Table 1,2 & 3) Deterioration of starch ad sugar contents in termialia beherica and terminalia chebula was studied (7) reduction in starch content observed by (5) deterioration of Radish and Arhar seed in storage due to aspergillus flavus was studied (6&11).

Packing wise biodeterioration of the phytoconstituent was found high in paper and cloth packings, highest microbial load was found in gunny bag since gunny bags re made of jute fiber which of botanical origin cellulytic compounds and are of hygroscopic in nature. Minimum load was recorded in polythene packing. Because polythene did not permit the growth of micro organisms and found much more effective in controlling th3e loss of the phytoconstituents, since results of polythene packing were only expected.

In the light of above it is necessary that suitable steps should be taken for the storage of herbal drugs so as to prevent the growth of microbes on them. It may be advisable to treat the herbal drugs in the warehouse wit antimicrobial non hazourdous agents to preserve for a long period. Studies on biodetrioration of phytoconstituents specially the medicinally active principles in
crude samples should be undertaken on a large scale.

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| PACKING USED       | TOTAL NO OF MICRO FLORA | PERIODICAL CHANGES IN THE TOTAL NO OF FUNGAL SP TOTAL PATHOGENIC BACTERIAL SPECIES AND SPC DURING STORAGE PERIOD (JAN TO DEC 1991) |
|--------------------|-------------------------|----------------------------------------------------------------------------------------------------------------------------------|
|                    |                         | 1989 | 1990 | 1991 |
|                    |                         | DEC | APR | AUG | DEC | APR | AUG | DEC |
| 1. UNPACKED (CONTROL) | (I) TOTAL NO OF FUNGAL SP | 6   | 14  | 22  | 15  | 7   | NIL | NIL |
|                    | (II) TOTAL PATH., BAC, SP | 1X10ⁱ | 17 X10¹ | 56 X10¹ | 7.4 X10¹ | 5.4 X10¹ | 1.6 X10¹ | NIL |
|                    | (III) SPC               |     |     |     |     |     |     |     |
| 2. CLOTH            | (I) TOTAL NO OF FUNGAL SP | 16  | 3   | 22  | 19  | 9   | 11  | NIL |
|                    | (II) TOTAL PATH., BAC, SP | 3   | 18 X10¹ | 68X10¹ | 18 X10¹ | 14 X10¹ | 9.4 X10¹ | NIL |
|                    | (III) SPC               |     |     |     |     |     |     |     |
| 3. POLYTHENE        | (I) TOTAL NO OF FUNGAL SP | 5   | NIL | 14  | 3   | 1   | NIL | NIL |
|                    | (II) TOTAL PATH., BAC, SP | 2X10¹ | NIL | 12 X10¹ | 2 X10¹ | NIL | NIL | NIL |
|                    | (III) SPC               |     |     |     |     |     |     |     |
| 4. PAPER            | (I) TOTAL NO OF FUNGAL SP | 17  | 1   | 20  | 17  | 8   | 11  | 2   |
|                    | (II) TOTAL PATH., BAC, SP | 2X10¹ | 3   | 71 X10¹ | 2   | 18.9 X10¹ | 16.4 X10¹ | 10.5 X10¹ |
|                    | (III) SPC               |     |     |     |     |     |     |     |
| 5. GUNNY BAG        | (I) TOTAL NO OF FUNGAL SP | 19  | 2   | 29  | 21  | 10  | 12  | 4   |
|                    | (II) TOTAL PATH., BAC, SP | 24X10¹ | 3   | 83 X10¹ | 3   | 31 X10¹ | 20 X10¹ | NIL |
|                    | (III) SPC               |     |     |     |     |     |     |     |
| 6. TREATED (GAMMA) (RADIATION) | (I) TOTAL NO OF FUNGAL SP | NIL | NIL | NIL | NIL | NIL | NIL | NIL |
|                    | (II) TOTAL PATH., BAC, SP | NIL | NIL | NIL | NIL | NIL | NIL | NIL |
|                    | (III) SPC               | NIL | NIL | NIL | NIL | NIL | NIL | NIL |
## Table -2
EFFECT GAMMA RADIATION AND DIFFERENT PACKINGS USED FOR STORAGE OF THE DRUG BAHERA FRUIT (TERMINALLA BELERICA ROXB) ON DURING STORAGE PERIOD

| S.no | Packing Used     | % of protein before packing of the drug in Dec 86 | 1990 | 1991 | Total loss in % during the storage period (24 months) |
|------|------------------|-----------------------------------------------|------|------|---------------------------------------------------|
|      |                  |                                              | APR  | AUG  | DEC      | APR  | AUG  | DEC  |                                                |
| 1.   | Unpacked (control) | 19.19                                       | 17.775 | 14.02 | 12.82 | 11.766 | 11.66 | 11.62 | 39.45                                        |
| 2.   | Cloth            | 16.0                                        | 13.5  | 12.0  | 11.61 | 11.00  | 10.60 |        | 44.70                                        |
| 3.   | Polythene        | 18.0                                        | 17.0  | 16.71 | 16.0  | 15.77  | 15.41 |        | 19.70                                        |
| 4.   | Paper            | 15.9                                        | 13.3  | 11.5  | 10.5  | 10.31  | 10.31 |        | 47.84                                        |
| 5.   | Gunny bag        | 14.0                                        | 12.03 | 10.15 | 9.12  | 8.78   | 8.78  |        | 57.69                                        |
| 6.   | Treated (Gamma)  | 19.0                                        | 19.01 | 18.99 | 19.0  | 18.99  | 18.99 |        | 1.04                                         |
### Table 3

**Effect of Gamma Radiation and Different Packings Used for Storage of the Drug Bahera Fruit (Terminalia belerica Roxb) on During Storage Period**

Loss in the protein content of the drug bahera fruit in % on dried basis (Jan 90 to Dec 91)

| S.no | Packing Used         | % of protein before packing of the drug in Dec 86 | 1990  | 1991  | Total loss in % during the storage period (24 months) |
|------|----------------------|-----------------------------------------------|-------|-------|---------------------------------------------------|
|      |                      |                                               | APR   | AUG   | DEC      | APR   | AUG   | DEC      |                                                   |
| 1.   | Unpacked (control)   | 23.24                                         | 23.00 | 22.89 | 22.86    | 22.84 | 22.84 | 22.82    | 1.81                                               |
| 2.   | Cloth                |                                               | 22.95 | 22.85 | 22.83    | 22.83 | 22.81 | 22.78    | 1.98                                               |
| 3.   | Polythene            |                                               | 23.1  | 23.0  | 22.98    | 22.98 | 22.94 | 22.94    | 1.29                                               |
| 4.   | Paper                |                                               | 22.94 | 22.83 | 22.81    | 22.80 | 22.79 | 22.74    | 2.15                                               |
| 5.   | Gunny bag            |                                               | 22.90 | 22.81 | 22.78    | 22.74 | 22.74 | 22.70    | 2.32                                               |
| 6.   | Treated (Gamma)      |                                               | 23.24 | 23.22 | 23.22    | 23.23 | 23.22 | 23.20    | 0.172                                              |
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